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# Monograph on Alternaria Diseases of Crucifers

**Saskatoon Research Centre**

**Canada**



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The images represent the Research Branch's objective: to improve the long-term competitiveness of the Canadian agri-food sector through the development and transfer of new technologies.

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*Conception par le Service aux programmes de recherches.*



Gerhard Lakon

# Monograph on Alternaria Diseases of Crucifers

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Technical Bulletin 1994-6E

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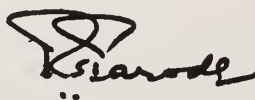
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## FOREWORD

The brassicas are an important group of oilseed crops, constituting almost 13.2% of the world's edible oil requirement. These are: *B. campestris*, *B. juncea*, *B. napus* and *B. carinata*. Together, they occupy about 14 million hectares of area with an annual production of 24.61 million tons. These have wide adaptability and are often grown under varied agroclimatic conditions throughout the world. Both area and production of these crops have increased substantially during the last two decades. It is encouraging that the total production has almost trebled during 1971 to 1990.

Considerable potential exists for improving both the production and productivity of oilseed brassicas through breeding improved varieties that are resistant to both biotic and abiotic stresses. Among biotic factors, alternaria, white rust, downy mildew and aphids are serious diseases and pests. Among these, alternaria diseases are causing heavy yield losses annually. Presently, there exists four species of *Alternaria* viz., *A. brassicae* (Berk.) Sacc., *A. brassicicola* (Schwein.) Wiltsh., *A. raphani* Groves and Skolko, and *A. alternata* (Fr.) Kiessl.

For evolving an effective strategy for the management of *Alternaria*, it is necessary that a review of available literature relating to distribution, taxonomy, physiological specialization, host range, disease cycle, pathogenesis, host-parasite interactions, yield losses, techniques for germplasm screening, infection detection and integrated disease management practices is available at one place. Such a comprehensive document is presently lacking. Accordingly, it is encouraging that Dr. P.R. Verma, Plant Pathologist (Oilseeds), Agriculture and Agri-Food Canada, Saskatoon Research Centre, Saskatoon, Saskatchewan, Canada and Dr. G.S. Saharan, Professor, Department of Plant Pathology, C.C.S. Haryana Agricultural University, Hisar, Haryana, India, have jointly undertaken an initiative to bring out this useful "Monograph on *Alternaria* diseases of Crucifers", after scanning more than 900 references which they published in 1993 in the form of a bibliography. I congratulate them for this endeavour. I firmly believe that this monograph will be of immense help to the scientists, teachers, students, extension specialists, and all those who are interested in the production of crucifer crops.



August, 1994

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## PREFACE

The aim of this monograph on *Alternaria* Diseases of Crucifers is to present a comprehensive compilation of the most recent information available on fundamental and applied knowledge of *Alternaria* species infecting Brassicaceae crops and weeds. The four species of *Alternaria* viz., *A. brassicae*, *A. brassicicola*, *A. raphani* and *A. alternata* are the most widely distributed and cause severe quantitative and qualitative yield losses in all regions of the world where these crops are grown. The major hosts of these pathogens are *Brassica* crops grown for high quality edible (rapeseed-mustard, canola and other rapes) and industrial oil (*Crambe*), common vegetables (cabbage, cauliflower, radish, kohlrabi, broccoli, brussels sprouts, kales, and other *Brassica* vegetables), and weeds. For convenience of the readers and coherence of the text, the information has been arranged into 16 sections and 45 sub-sections. The treatment has been of the disease, its symptoms on different hosts, geographic distribution, yield losses and disease assessment methods; the pathogen's taxonomy, variability, sporulation, perpetuation and spore germination; host-parasite interactions in the form of seed infection, host range, disease cycle, process of infection and pathogenesis, epidemiology, fine structure, biochemical changes and phytotoxins; host defense mechanisms; techniques to study host-parasite relationships and management practices related to cultural, chemical, biological control and host resistance. The last section deals with gaps in our understanding and management of these diseases and offers suggestions for future research priorities.

We believe that this monograph will be useful to research scientists, teachers, extension specialists, students and others who are dealing with Brassicaceae crops.

August, 1994

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During the preparation of this monograph, several of the senior authors' colleagues, including late Dr. Ron Howarth, Dr. P.A. O'Sullivan as Director, and Dr. Keith Downey as former, and Dr. Gerhard Rakow as present Chairman of the Oil-seed Section, at Saskatoon, have made valuable suggestions and have been very supportive of the work. The authors also thank Dr. C.F. Hinks, Research Scientist, Agriculture and Agri-Food Canada, Research Centre, Saskatoon, and Dr. J.P. Tewari, Professor, Department of Plant Science, University of Alberta, Edmonton, Canada, for critically reviewing the manuscript. Here, we also wish to thank many people at Headquarters Library, Agriculture Canada, Ottawa, for providing countless photocopies, and to the Translation Bureau, Department of Secretary-of-State for translation of a series of foreign language papers.

The second author also thanks Vice-Chancellor, C.C.S. Haryana Agricultural University, Hisar, India, for permitting him to spend two months in the senior author's laboratory to co-author this publication.

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## 1. INTRODUCTION

The most common and destructive diseases of Brassicaceae crops worldwide are those caused by four species of *Alternaria* viz., *A. brassicae* (Berk.) Sacc., *A. brassicicola* (Schwein.) Wiltsh., *A. raphani* Groves and Skolko, and *A. alternata* (Fr.) Keissl. Although attack by *Alternaria* at the seedling stage can lead to death of young plants, infection on leaves, stems and siliques generally results in heavy losses in seed yield and quality.

*Brassica* oilseed crops occupy over 14 million hectares of the world's agricultural lands and provide over 13.2% of the world's edible vegetable oil (Downey and Röbbelen, 1989). Their ability to survive and grow at low temperatures enables the oilseed brassicas to be successfully cultivated in cool agricultural regions, at high elevations, and as winter crops in the subtropics.

The small round seeds of *Brassica* oilseed crops contain over 40% oil on a dry weight basis, and after oil extraction, provide a meal containing over 40% high quality protein. In the western countries the meal is utilized exclusively as a feed for livestock and poultry, but in many Asian countries it is used as an organic fertilizer for field crops.

The Brassicaceae family to which the genus *Brassica* belongs, contains many important crop plants yielding high quality edible and industrial oils, common vegetables and weeds. Based on the evidence that some vegetable types were in common use in the Neolithic age (Chang, 1968; Hyams, 1971), and direct reference to oilseed rape and mustard in the ancient Indian Sanskrit writings of 2000-1500 BC (Singh, 1958), the *Brassica* vegetables and oilseeds may well have been among the earliest plants domesticated by man. Greek, Roman and Chinese writings of 500-200 BC also mention these crops and their medicinal value (Prakash and Hinata, 1980). Oilseed rape was introduced in China and Japan around the time of Christ (Haugen and Stefansson, 1983). Although its cultivation was started in the 13th century in Europe, its industrial use was not widespread until its superior qualities as a lubricant oil were recognized (Shahidi, 1990). Its use as an edible vegetable oil in western countries is very recent.

Unlike most other oilseeds, rapeseed comes from several species belonging to the genus *Brassica* (Shahidi, 1990). These species include *B. napus* L., *B. rapa* L. (= *B. campestris* L.) and *B. juncea* (L.) Czern. & Coss. which are known as rape, turnip rape and leaf mustard, respectively. Common names which have been used for *B. napus* are rape, rapeseed, oil rape, colza, oilseed rape, swede rape and Argentine rape, for *B. rapa* are rapeseed, oil turnip, and Polish rape, and for *B. juncea* are brown mustard, oriental mustard, Indian mustard and rapeseed. In China, all three species are grown but winter-grown rape constitutes the major source of rapeseed. In India, turnip rape and mustard may be considered rapeseed, and in North America and Europe, rape and turnip rape are regarded as rapeseed. The other oilseed crops of the Brassicaceae include *B. rapa* L. var. toria (turnip rape, toria), *B. rapa* L. var. brown sarson (turnip rape, brown sarson), *B. rapa* L. var. yellow sarson (turnip rape, yellow sarson), *B. nigra* (L.) Koch (Black mustard), *B. hirta* Moench (= *Sinapis alba* L.) (white mustard), *B. carinata* A. Braun



(Abyssinian mustard, Ethiopian mustard), *B. tournefortii* Gouan (wild turnip), *Eruca sativa* Mill. (= *E. vasicaria* spp. *sativa* (Mill.) Thell.) (Garden rocket, taramira), *Camelina sativa* Crantz. (false flax, Dutch flax, gold-of-pleasure), *Crambe abyssinica* Hochst. ex. O.E. Schulz and *C. hispanica* L.

The traditional varieties of rapeseed that are being grown in the Asian countries contain 22-60% erucic acid in their oil, and also high percentage of glucosinolate in their defatted meal. While the presence of erucic acid compromised the nutritional value of oil, the presence of glucosinolate limited the feeding value of the meal. More recent Canadian varieties (*B. napus* and *B. rapa*) have been genetically modified to contain low erucic acid and low glucosinolates. The name "canola" was adopted in 1979 to apply to Canada to all "double low" cultivars. The term canola thus refers to a rapeseed cultivar that contains less than 30  $\mu\text{mol/g}$  of one or any combination of the four known aliphatic glucosinolates (gluconapin, progoitrin, glucobrassicinapin, and napoleiferin) in its defatted meal, and less than 2% of the fatty acyl content of the oil is erucic acid. Canada recently has also developed canola type *B. juncea* cultivars.

The main groups of cultivated *Brassica* vegetables are: kales (*B. oleracea* L. var. *acephala*) including kitchen kale, green kale, dwarf Siberian kale, narrow stem kale, collards, trochunda; cabbages (*B. oleracea* L. var. *capitata*, var. *sabauda*, var. *bullata*) including headed cabbages, brussels sprouts, savoy cabbage; kohlrabi (*B. oleracea* L. var. *gongylodes*); inflorescence kales (*B. oleracea* L. var. *botrytis*, var. *italica*) including cauliflower, broccoli, sprouting broccoli; branching bush kales (*B. oleracea* L. var. *fruticosa*) including cow-kale, borecole, thousand-headed kale; Chinese kale (*B. alloglabra* L.) (Snogerup, 1980); and radishes (*Raphanus sativus* L.).

*Crambe* is a newly emerging oilseed crop with increasing commercial acreage in the United States, Poland and some other countries of the world. *Crambe* oil is a potential raw material for rubber and plastic industries. The prospective species for cultivation are *Crambe abyssinica*, *C. hispanica*, and some other annuals.

## 2. THE DISEASE

### a. Synonyms

The diseases of Brassicaceae crops caused by four species of *Alternaria* are known by many names: alternaria leaf spot, alternaria pod spot, alternaria leaf and pod spot, alternaria blight, alternaria leaf blight, alternaria pod blight, alternaria leaf and pod blight, alternaria dark leaf spot, black spot, brown spot, blight pod spot, stem streak, seed rot, siliquae mould, black mould, grey leaf spot, grey leaf mould of rapeseed - mustard and crucifers, rape black, brasicaceous black, black spot of cabbage, brown rot of cauliflower, alternaria storage rot of vegetables, alternaria blight of vegetables, alternaria sooty spot, black pod blotch, brown rot, curd blight, curd drying, root and foot rot and damping-off (Anonymous, 1985a-d; Czyżewska, 1969, 1970, 1971; Ellis, 1968a,b; Kolte, 1985a,b; Leppik, 1973; Neergaard, 1945; Petrie, 1975; Pound, 1946; Sherf and Macnab, 1986; Subramanian, 1971; Tewari, 1985; Van Schreven, 1953; Walker, 1952; Weber, 1973; Wiltshire,



1947; Yoshii, 1933).

## **b. Symptoms**

i) **Rapeseed - Mustard:** On oil-yielding Brassica crops all three species of *Alternaria* (*A. brassicae*, *A. brassicicola* and *A. raphani*) are reported to cause symptoms in the seedling stage on cotyledons and in the adult stage on leaves, leaf petiole, stem, inflorescence, siliquae and seeds (Fig. 1). There may be variations in shape, size, colour and intensity of lesions on different host tissues and species under different agro-ecological zones. In general, lesions produced by *A. brassicae* are grey compared to black sooty velvety lesions produced by *A. brassicicola*. All three species produce distinct lesions with yellow halos around them. The disease first appears on the cotyledons and hypocotyls in the form of small light brown lesions which soon turn black due to the appearance of spore masses (under humid conditions) and act as a source of infection for the other parts of the plant. Damping off of rapeseed seedlings due to *A. brassicicola* is common in Finland (Tahvonon, 1979). The initial infection on the lower leaves starts as minute brown to blackish lesions which multiply rapidly and later spread to the upper leaves, stem and siliquae. On the leaves, lesions may vary in size from 1–20 mm. In some *Brassica* species formation of concentric rings in the lesions and a zone of yellow halo around the lesions is very prominent (Kadian and Saharan, 1983). Several lesions on the leaves unite to cause blighting and defoliation under humid weather. The lesions on the stem are at first linear and then expand but remain usually elongated with pointed ends. In severe attacks, the upper part of the stem and siliquae wither. Siliquae may show sunken, dark brown to black circular lesions. Deep lesions on the siliquae cause infection in the seed. On siliquae of *B. rapa* (toria, yellow and brown sarson) lesions are more prominent, enlarged and dark brown to black in colour than on *B. juncea* where they are light coloured with distinct grey centers. The diseased seeds just beneath the black spot are small, shrivelled, grey to brown in colour. In years of severe outbreaks, infection of the stem may be sufficiently intense to cause the whole plant to wither before many of the pods have matured or even formed (Kolte, 1985a,b; Saharan and Chand, 1988; Vasudeva, 1958). Detailed symptomatology of *Brassica* species infected with *A. brassicae* has been worked out by Conn *et al.* (1990) and Kadian and Saharan (1983). On Brassicaceae, the lesions on leaves due to *A. brassicicola* are sooty black, velvety and copiously covered with black conidiophores and conidia, whereas those caused by *A. brassicae* are gray, dense and sparsely covered with brown conidiophores and conidia (Changsri, 1961); the lesions caused by *A. raphani* are small with raised margins and are surrounded by a yellow translucent halo.

In *A. brassicae* infections in rapeseed, the chloroplasts are more severely affected than the mitochondria. Changes in chloroplasts include swelling and eventual disintegration. The mitochondria show swelling of intracrystal spaces but without disintegration. In severely affected cells, vesiculated plasma membranes with electron-dense deposits, intact grana from disintegrated chloroplasts, and mitochondria with swollen intracrystal spaces are recognizable (Tewari, 1991b).



Fig. 1. *Alternaria brassicae* infection on *Brassica rapa* (a) leaf; (b) siliquae; and (c) seeds (Courtesy: Conn, Tewari and Awasthy, 1990).



*Alternaria alternata* causes root rot of rape (*B. rapa*) in Canada (Berkenkamp and Vaartonov, 1972) and root and foot rot of *Eruca sativa* in India (Bhargava *et al.*, 1980). The first evidence of the disease in the fields is poor germination of seeds. The infected cotyledons first become chlorotic and then necrotic. The necrotic lesions extend on the stem up to 6–7 cm from the soil level. The root system of severely infected plants is completely destroyed (Bhargava *et al.*, 1980).

ii) **Crambe:** *Alternaria brassicae* infects mainly leaves and stems, *A. brassicicola* is a major pathogen of stem and siliquae, whereas *A. alternata* infects all above ground plant parts; *A. raphani* has not been recorded on this crop (Czyzewska, 1971). In general, symptoms caused by all the three species are similar. *Alternaria*-infected seeds when sown cause pre- and post-emergence damping-off of seedlings. In dry seasons, diseased cotyledons turn yellow, shrivel and dehisce prematurely. During humid weather initial spots on the infected cotyledons are small, dark brown which quickly become large and cover all the tissues. Such cotyledons generally become slimy with mycelium, conidiophore and conidia leading to quick seedling death.

Leaf lesions are round (0.5–2 mm dia.) slightly depressed with a smooth surface and brown to dark brown in colour. The tissues around the spots turn lighter in colour and then become yellow. Sometimes, the brown dead tissues in the central part of the spot crumble, and fray at the leaf edge in the form of a 0.5 mm width brown tissue border. The spots caused by *A. brassicae* are larger (1–10 mm), and appear isolated with visible infection centre. The concentric rings in the lesion are more sparsely arranged usually at a distance of 1–2 mm. Two to three rings appear on one spot. Larger spots are lighter brown in colour and have a slight violet tint (Fig. 2).

Spots on the infected stems are dark brown to black, mostly elongated in the form of streaks and somewhat recessed. In damp weather infected stems become constricted causing the seedlings to topple (Fig. 2). The infected collar region appears recessed with elongated black to olive lesion of up to 2 cm encircling the stem partially or completely.

On siliquae initial lesions are small (0.3–3 mm), round, olive brown in colour later turning blackish-brown after sporulation. Infected siliquae are small, deformed and produce small sized, discoloured, shrivelled seeds, which are non-viable (Czyzewska, 1969, 1971; Holcomb and Newman, 1970; Leppik, 1973).

iii) **Garden Stock (*Matthiola incana*):** The initial symptoms appear on the lower leaves first, which gradually spread to upward onto the leaves, stem and flowers. The lesions first appear round, then elongate to 3–15 mm in size. Older lesions are pale or greyish green with concentric zones with brown centres. Such lesions become dark brown to black during sporulation. On stem and flowers, lesions with water-soaked margins are also common (Davis *et al.*, 1949).

**iv) Vegetable Crops:** The symptoms produced by the two species (*A. brassicae* and *A. brassicicola*) are similar and often indistinguishable. Seed infection by either fungus may cause severe damping-off or a stunting of young plants. On infected seedlings the fungi produce dark brown necrotic areas on the cotyledons and similar coloured streaks on the hypocotyl. On older plants all above-ground parts are attacked including leaves, stems, brussels sprouts buttons, cauliflower curds, inflorescence and siliquae including seed in seed crop. On leaves, symptoms first appear as minute dark brown to black spots each surrounded by a halo of chlorotic tissues. Leaf spots that vary in size from pinpoints to 2 to 3 inches in diameter are common on old lower leaves. The enlargement of the spots may be in concentric circles. Older lesions are circular, often zonate with a papery, thin centre and may be covered with a mat of spores which are yellow in the case of *A. brassicae*, and dark olive brown in the case of *A. brassicicola*. The centres of the lesions may fall out to give a shot-hole effect. Severe defoliation by *A. brassicae*, has been reported in some cultivars of stubble turnips. The lesions are linear on stem, petiole and siliquae (Chupp, 1925; Chupp and Sherf, 1960).

Cauliflower and broccoli heads show a browning, beginning at the margin of the individual flower and flower cluster. On plants grown for seed, dark necrotic lesions occur on the main axis, the inflorescence branches and on the siliquae. These lesions coalesce rapidly and cause premature ripening and splitting of the siliquae and may result in high levels of seed infection. Infected seeds are small, shrunken, discoloured or covered with fungal growth and have low viability. Cankers may form just below nearly mature cabbage heads and result in stump rot and death of plants.

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When turnips and rutabaga foliage is infected, the roots may also become infected and develop symptoms, especially after being placed in storage. The leaf spots are nearly circular, often zonate, and are various shades of brown to black. Dark spores may cover the spot if temperature is high during storage (Sherf and Macnab, 1986). *A. raphani* is common on radish plants kept for seed purpose. Leaf lesions are raised, spherical to elliptical and up to 1 cm in diameter. Black sporulation may be seen on the lesions. The centre soon dries and may drop out (Singh, 1987). The natural infection of radish seed with *A. raphani* may result in a lack of germination, a pre- or post-emergence blight, the appearance of distinctive lesions of cotyledons and hypocotyls, the presence of scablike lesions on table radish and in the spotting and blighting of leaves, stalks and siliquae (Atkinson, 1950).



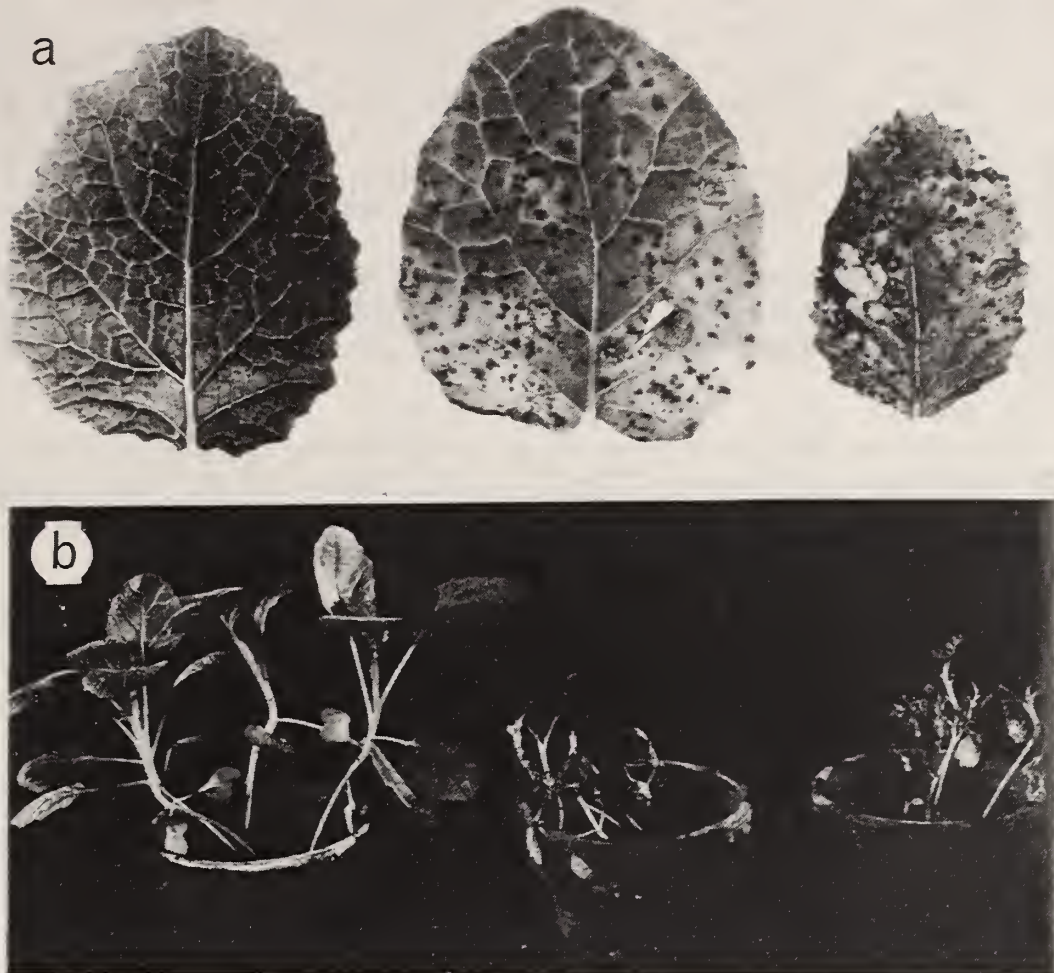


Fig. 2. *Alternaria brassicicola* infection on *Crambe abyssinica* (a) leaves; and (b) seedlings (Courtesy: Kilpatrick, 1976).

### c. Geographical Distribution

Amongst the different *Alternaria* species reported to infect brassicaceous hosts, *A. alternata* is considered as a weak pathogen being an extremely common saprophyte found on many kinds of plants and other substrata. It is cosmopolitan and reported to be widespread in its occurrence on all kinds of brassicaceous plants including oil-yielding crops like rapeseed-mustard, crambe and Brassica vegetables (Ellis, 1971; Verma and Saharan, 1993).

*Alternaria brassicae* (CMI map 353) and *A. brassicicola* (CMI map 457) have been reported from almost every continent on brassicaceae hosts (Anonymous, 1969). *A. brassicae* is considered most destructive on oil-yielding brassicas and both are common on vegetable crucifers. They are known to occur in Africa, Argentina, Australia, Austria, Bangladesh, Brazil, Britain, Bhutan, Bulgaria, Burma, Canada, Chile, China, Cyprus, Czechoslovakia, Denmark, England, Ethiopia, Finland, France, Germany, Ghana, Guinea, Holland, Hong Kong, Hungary, India, Ireland,

Italy, Jamaica, Japan, Kenya, Libya, Malawi, Malaya, Mauritius, Morocco, Mozambique, Nepal, Netherland, New Guinea, New Zealand, Nicaragua, Nigeria, Norway, Pakistan, Phillipines, Poland, Rhodesia, Romania, Russia, Sabah, Saudi Arabia, Scotland, Sierra Leone, Singapore, Spain, South Africa, Sri Lanka, Sudan, Sweden, Switzerland, Taiwan, Tanganyika, Tanzania, Trinidad, Turkey, Uganda, USA, USSR, and Zambia.( Anonymous, 1980, 1981, 1984, 1985a-d; Ellis, 1971; Kolte, 1985a; Saharan, 1992b; Verma and Saharan, 1993).

*Alternaria cheiranthi* (Lib.) Bolle. is common on wallflowers and occasionally recorded on other plants, especially on Brassicaceae. It is recorded from Belgium, Denmark, France, Germany, Great Britain, Holland, Ireland, and Italy (Ellis, 1971).

*Alternaria raphani*, which occurs on various brassicaceous hosts have been recorded from Canada, Denmark, Egypt, Greece, India, Japan, Netherlands and USA (Ellis, 1971). It is most common on radish, but also occurs on other *Brassica* vegetables and oil-yielding crops (Verma and Saharan, 1993).

*Alternaria alternata* has been reported from Canada, Denmark, France, Poland, Latvian SSR, Leningrad USSR and Maryland USA (Czyzewska, 1969, 1970; Leppik, 1973; Neergaard, 1945).

The chronological records of *Alternaria* species causing diseases of brassicaceous hosts reported from various countries of the world are given in Table 1. The names of various hosts in this table are as reported in the original papers.

Table 1. Records of *Alternaria* species on Brassicaceae (Verma and Saharan, 1993)

| <i>Alternaria</i><br>species                | Host                                      | Disease                  | Location     | Recording<br>year | Reference                       |
|---|---|--------------------------|--------------|-------------------|---------------------------------|
| <i>A. alternata</i><br>( <i>A. tenuis</i> ) | cauliflower                               | Pox                      | Italy        | 1932              | Verona, 1932                    |
|   | cabbage                                   | Seed crop,<br>Black spot | SSSR         | 1959              | Nelen, 1959                     |
|   | <i>B. campestris</i><br>& <i>B. napus</i> | Black spot               | Canada       | 1963              | Taber &<br>Vanterpool, 1963     |
|   | <i>Brassica</i> spp.                      | Black spot               | Canada       | 1967              | Conners, 1967                   |
|   | <i>Eruca sativa</i>                       | Root and<br>foot rot     | India        | 1979              | Bhargava <i>et al.</i> , 1980   |
|   | <i>C. abyssinica</i>                      | Black spot               | Poland       | 1969              | Czyzewska, 1969                 |
|   | <i>B. campestris</i>                      | Root rot                 | Canada       | 1971              | Berkenkamp &<br>Vaartnov, 1972  |
|   | Garden rocket                             | Black spot               | Saudi Arabia | 1978              | Sheir <i>et al.</i> ,<br>1981   |
|   | <i>Brassica</i> spp.                      | Black spot               | USA          | 1978              | Babadoost &<br>Gabrielson, 1979 |

|  |                          |                                  |                         |      |                            |
|--|--------------------------|----------------------------------|-------------------------|------|----------------------------|
| <i>A. brassicae</i><br>( <i>Macrosporium brassicae</i> ;<br><i>A. herculea</i> ) | Radish                   | Leaf & pod blight                | India                   | 1980 | Suhag <i>et al.</i> , 1983 |
|  | Cabbage                  | Leaf spot                        | USA                     | 1909 | Fawcett, 1909              |
|  | Collards                 | Black mould                      | USA                     | 1913 | Higgins, 1917              |
|  | Cauliflower              | Leaf spot & brown rot            | USA                     | 1918 | Weimer, 1924               |
|  | Cabbage                  | Black leaf spot                  | USA                     | 1918 | Harter & Jones, 1923       |
|  | Cabbage                  | Leaf spot                        | USA                     | 1922 | Chupp, 1923                |
|  | Cabbage                  | Leaf spot                        | USA                     | 1922 | Milbraith, 1922            |
|  | Cabbage & Radish         | Leaf spot                        | Russia                  | 1922 | Estifeyeff, 1925           |
|  | Cabbage                  | Black leaf spot                  | Trinidad                | 1922 | Stell, 1922                |
|  | <i>Brassica</i> spp.     | Leaf spot                        | Holland                 | 1924 | Bolle, 1924                |
|  | <i>Brassica</i> spp.     | Black leaf speck                 | USA                     | 1926 | Nelson, 1926               |
|  | Cabbage                  | Leaf spot                        | China                   | 1926 | Porter, 1926               |
|  | Brassicaceous vegetables | Leaf spot                        | USA                     | 1926 | Weimer, 1926               |
|  | <i>B. pekinensis</i>     | Leaf spot                        | USA                     | 1927 | Gardner, 1929              |
|  | <i>Brassica</i> spp.     | Leaf spot                        | Dominican Republic      | 1927 | Ciferri & Gonzalez, 1927   |
|  | Cauliflower              | Brown rot                        | USA                     | 1927 | Walker, 1927               |
|  | Cauliflower              | Black leaf spot                  | Italy                   | 1928 | Gardner, 1929              |
|  | Cauliflower              | Black spot                       | Colombia                | 1929 | Toro, 1929                 |
|  | Cabbage                  | Leaf spot & storage rot          | North Caucasus          | 1930 | Kikoina, 1930              |
|  | Cabbage                  | Seed infection                   | Holland                 | 1930 | Voisenat, 1930             |
|  | Cabbage                  | Leaf spot                        | Italy                   | 1931 | Pollacci, 1932             |
|  | Cabbage & Radish         | Leaf spot                        | Japan                   | 1933 | Yoshii, 1933               |
|  | Brassicaceous Vegetables | Leaf spot                        | U.K.                    | 1933 | Moore, 1944                |
|  | Cauliflower & Broccoli   | Black leaf spot & grey leaf spot | Canada                  | 1934 | Connors, 1935              |
|  | Chinese Cabbage          | Leaf spot                        | USA                     | 1934 | Davis, 1934                |
|  | <i>Brassica</i> spp.     | Seed infection                   | Germany                 | 1934 | Juhans, 1934               |
|  | Brassicaceous Vegetables | Leaf spot                        | Trinidad<br>Phillipines | 1934 | Fajardo & Palo, 1934       |
|  | <i>Matthiola incana</i>  | Leaf spot                        | Great Britain           | 1935 | Ware, 1936                 |
|  | Turnip                   | Root rot                         | USA                     | 1935 | Chupp, 1935                |
|  | Cabbage                  | Leaf spot                        | Morocco                 | 1937 | Malencon & Delecluse, 1937 |
|  | <i>B. oleracea</i>       | Leaf spot                        | Africa                  | 1937 | Roger & Mallamaire, 1937   |
|  | Colza & Rape             | Leaf spot                        | Germany                 | 1938 | Klemm, 1938                |
|  | Cauliflower head         | Grey rot                         | Brazil                  | 1938 | Arruda, 1938               |
|  | Horse Radish             | Leaf spot                        | Germany                 | 1938 | Boning, 1938               |



|   |                        |               |      |                                 |
|---|------------------------|---------------|------|---------------------------------|
| <i>Iberis umbellata</i>   | Leaf spot              | Denmark       | 1938 | Neergaard, 1939                 |
| Horse radish  | Leaf spot              | USA           | 1940 | Kadow & Anderson, 1940          |
| Cabbage   | Leaf spot              | China         | 1940 | Teng, 1940                      |
| Turnip  | Leaf spot              | Australia     | 1941 | Anonymous, 1941                 |
| Cabbage   | Black spot             | USA           | 1941 | Godfrey, 1941                   |
| Cabbage   | Storage rot & Ballhead | USA           | 1942 | Myers, 1942                     |
| Cabbage & Cauliflower   | Leaf spot              | USA           | 1942 | Snyder & Baker, 1943, 1945      |
| Radish & Turnip   | Leaf spot              | Argentina     | 1943 | Marchionatto, 1947              |
| Turnip  | Leaf spot              | U.K.          | 1943 | Moore, 1948                     |
| Colza & White mustard   | Black spot             | Sweden        | 1944 | Bjorling, 1944                  |
| Cabbage   | Seed infection         | USA           | 1944 | Anonymous, 1944                 |
| Cauliflower & Broccoli  | Head browning          | USA           | 1944 | Rangel, 1945                    |
| Cabbage & Turnip  | Brown rot              | Tanganyika    | 1945 | Wallace & Wallace, 1945         |
| Turnip, Kohlrabi  | Leaf spot              | Norway        | 1945 | Jorstad, 1945                   |
| Cabbage & Radish  | Leaf spot              | Denmark       | 1945 | Neergaard, 1948                 |
| Brassicaceous vegetable, <i>M. incana</i> & <i>I. umbellata</i> | Leaf spot              | Denmark       | 1945 | Neergaard, 1948                 |
| Broccoli  | Dark leaf spot         | U.K.          | 1946 | Moore, 1948                     |
| Radish  | Leaf spot              | U.K.          | 1946 | Moore, 1948                     |
| Cabbage & Mustard   | Leaf spot              | Ceylon        | 1947 | Bond, 1947                      |
| Rape  | Seedling blight        | Canada        | 1948 | Vanterpool, 1950                |
| <i>C. maritima</i>  | Grey leaf spot         | France        | 1949 | Darpoux & Faivre-Amiot, 1949    |
| Cabbage, mustard & turnip                                       | Leaf spot              | Holland       | 1949 | Flik & Saaltink, 1950           |
| Turnip  | Leaf spot              | New Zealand   | 1949 | Brien & Dingley, 1953           |
| <i>Brassica</i> spp.  | Leaf spot              | Holland       | 1950 | Anonymous, 1951b                |
| <i>Lunaria annua</i>  | Leaf spot              | USA           | 1950 | Baker & Davis, 1950             |
| Cabbage   | Leaf spot              | Nyasaland     | 1950 | Gillman, 1952                   |
| Sugar beet  | Leaf spot              | USA           | 1950 | McFarlane <i>et al.</i> , 1954  |
| <i>Brassica</i> spp.  | Leaf spot              | India         | 1950 | Mehta <i>et al.</i> , 1950      |
| <i>B. napus</i>   | Dark leaf spot         | Holland       | 1950 | Van Schreven, 1953              |
| Turnip  | Leaf spot              | Kenya         | 1951 | Anonymous, 1951a                |
| Cabbage   | Leaf spot              | New Caledonia | 1951 | Bugnicourt <i>et al.</i> , 1951 |
| Turnip  | Leaf spot              | Sudan         | 1951 | Tarr, 1954                      |
| Chinese Cabbage   | Leaf spot              | USSR          | 1952 | Nelen, 1966                     |
| Candy Tuft  | Stem spot              | Tanganyika    | 1952 | Wallace, 1954                   |



|   |                           |                     |      |                                |
|---|---------------------------|---------------------|------|--------------------------------|
| <i>C. abyssinica</i>                                    | Grey leaf spot            | Latvian SSR         | 1954 | Leppik, 1973                   |
| <i>Brassica</i> spp.                                    | Dark leaf spot            | Ireland             | 1956 | McKay, 1956                    |
| Rape  | Grey leaf spot            | Canada              | 1956 | McDonald, 1959                 |
| Cabbage   | Seedling blight           | USSR                | 1956 | Tupenevich & Shirko, 1956      |
| Cabbage & Turnip  | Leaf spot                 | Nicaragua           | 1957 | Litzenberger & Stevenson, 1957 |
| Colza   | Black spot                | France              | 1958 | Louvet, 1958                   |
| Rape  | Siliquae & seed infection | Poland              | 1958 | Czyzewska, 1958                |
| Cabbage   | Leaf spot                 | Greece              | 1959 | Koleva-Sekutkovska, 1959       |
| Turnip & <i>Sisymbrium orientale</i>                    | Leaf spot                 | Australia           | 1959 | Anonymous, 1959                |
| Cabbage   | Leaf spot                 | Primorskil Kral SSR | 1959 | Nelen, 1959                    |
| <i>L. annua</i> & <i>L. rediviva</i>                    | Leaf spot                 | Rumania             | 1959 | Negru, 1959                    |
| Rape  | Grey leaf spot            | Canada              | 1961 | Downey & Bolton, 1961          |
| Turnip  | Root rot & leaf spot      | USA                 | 1961 | Ramsey & Smith, 1961           |
| Radish  | Leaf spot                 | Canada              | 1963 | Taber & Vanterpool, 1963       |
| <i>Thlaspi arvense</i> & <i>Lepidium</i> spp.           | Leaf spot                 | Canada              | 1966 | Petrie & Vanterpool, 1966      |
| <i>Sinapis alba</i>                                     | Seed infection            | Denmark             | 1967 | Jorgensen, 1967                |
| Radish  | Leaf spot                 | Rumania             | 1967 | Barbu & Dinescu, 1969          |
| <i>B. alboglabra</i>                                    | Leaf spot                 | Singapore           | 1969 | Seow & Lim, 1969               |
| <i>C. abyssinica</i>                                    | Grey leaf spot            | Poland              | 1970 | Czyzewska, 1970                |
| <i>C. abyssinica</i>                                    | Grey leaf spot            | USSR                | 1970 | Leppik, 1973                   |
| Rape  | Black spot                | Western Australia   | 1971 | Bokor, 1972                    |
| Radish  | Leaf spot                 | India               | 1977 | Rao, 1977                      |
| Turnip rape   | Black spot                | Spain               | 1979 | Romero & Jimenez, 1979         |
| Turnip  | Leaf spot                 | Bulgaria            | 1979 | Khristov, 1979                 |
| <i>B. napus</i>   | Leaf spot                 | Mexico              | 1980 | Ponce & Mendoza, 1983          |
| Rape  | Leaf spot                 | Sweden              | 1981 | Doughty <i>et al.</i> , 1991   |
| <i>Anagallis arvensis</i> , <i>Convolvulus arvensis</i> | Leaf spot                 | India               | 1982 | Saharan <i>et al.</i> , 1982   |
| Rape  | Leaf spot                 | U.K.                | 1982 | Anonymous, 1983                |
| <i>Brassica</i> spp.                                    | Dark leaf spot            | Ireland             | 1982 | Anonymous, 1983                |
| Charlock Weed   | Leaf spot                 | Ireland             | 1982 | Anonymous, 1983                |
| Cabbage,  | Curds spot                | U.K.                | 1982 | Anonymous, 1983                |

|                        |   |                            |              |      |                             |
|------------------------|---|----------------------------|--------------|------|-----------------------------|
| <b>A. brassicicola</b> | Brussels Sprouts<br>& Cauliflower Curds   |                            |              |      |                             |
|                        | <i>Brassica</i> spp.                      | Leaf spot                  | Ireland      | 1983 | Ryan <i>et al.</i> , 1984   |
|                        | Swede rape                                | Leaf spot                  | Russia       | 1983 | Vakhurusheva,<br>1983       |
|                        | <i>B. napus</i> &<br><i>B. campestris</i> | Leaf spot                  | Italy        | 1985 | Tosi & Zazzerini,<br>1985   |
|                        | Oilseed rape                              | Dark leaf<br>spot          | U.K.         | 1985 | Anonymous, 1985d            |
|                        | Candy tuft<br>( <i>Iberis</i> sp.)        | Leaf spot                  | India        | 1987 | Gurha & Dhar,<br>1987       |
|                        | <i>B. napus</i> &<br><i>B. carinata</i>   | Leaf spot                  | Ethiopia     | 1987 | Kidane & Bekele,<br>1986    |
|                        | Rape                                      | Dark leaf<br>spot          | Germany      | 1988 | Daebeler &<br>Amelung, 1988 |
|                        | Cabbage                                   | Leaf spot                  | Holland      | 1924 | Bolle, 1924                 |
|                        | Cabbage &<br>Cauliflower                  | Damping-off<br>& dark spot | Burma        | 1934 | Su, 1934                    |
|                        | Cauliflower                               | Inflorescence<br>blight    | Mauritius    | 1943 | Coombes &<br>Julien, 1949   |
|                        | Chinese<br>Cabbage                        | Leaf spot                  | Jamaica      | 1944 | Anonymous, 1945             |
|                        | <i>Godetia hybrida</i>                    | Seed<br>infection          | Denmark      | 1945 | Anonymous, 1946             |
|                        | <i>C. abyssinica</i>                      | Black spot                 | Denmark      | 1945 | Neergaard, 1945             |
|                        | Vegetable<br>Brassicas &<br>Weeds         | Leaf spot                  | Denmark      | 1945 | Neergaard, 1948             |
|                        | Cabbage, Cauli-<br>flower & kale          | Black spot                 | UK           | 1947 | Green, 1947                 |
|                        | Kohl-rabi                                 | Leaf spot                  | Ceylon       | 1947 | Bond, 1947                  |
|                        | Cabbage                                   | Leaf spot                  | Nyasaland    | 1950 | Gillman, 1952               |
|                        | Turnip,<br>Cauliflower &<br>Radish        | Leaf spot                  | Sudan        | 1951 | Tarr, 1951, 1954            |
|                        | Cabbage                                   | Leaf spot                  | New Zealand  | 1952 | Brien & Dingley,<br>1955    |
|                        | Cabbage,<br>Brussels<br>Sprouts & radish  | Leaf spot &<br>Blackening  | Germany      | 1952 | Stoll, 1952                 |
|                        | Cabbage &<br>Cauliflower                  | Damping-off                | Finland      | 1952 | Linnasalmi, 1952            |
|                        | Cabbage                                   | Seed<br>infection          | Turkey       | 1954 | Gobelez, 1956               |
|                        | <i>Brassica</i> spp.                      | Dark leaf<br>spot          | Ireland      | 1956 | McKay, 1956                 |
|                        | Cabbage                                   | Dark spot                  | SSSR         | 1959 | Nelen, 1959                 |
|                        | <i>B. rapa</i>                            | Leaf spot                  | Malaya       | 1963 | Jamil, 1966                 |
|                        | <i>Brassica</i> spp.                      | Leaf spot                  | Brunei       | 1963 | Johnston, 1964              |
|                        | Rape                                      | Leaf spot                  | Chile        | 1963 | Bertossi, 1963              |
|                        | Cabbage                                   | Leaf spot                  | Saudi Arabia | 1967 | Anonymous, 1967             |
|                        | <i>Brassica</i> spp.                      | Leaf spot                  | Canada       | 1967 | Connors, 1967               |
|                        | <i>C. abyssinica</i>                      | Black spot                 | Poland       | 1969 | Czyzewska, 1969             |
|                        | <i>C. abyssinica</i>                      | Black spot                 | USA          | 1969 | Holcomb &<br>Newman, 1970   |
|                        | <i>Brassica</i> spp.                      | Leaf spot                  | Sarawak      | 1972 | Kueh, 1972                  |
|                        | White Cabbage                             | Storage rot                | U.K.         | 1973 | Geeson, 1979                |

|                   |                            |   |              |      |                              |
|-------------------|----------------------------|---|--------------|------|------------------------------|
|                   | Crucifers                  | Leaf spot                               | India        | 1977 | Rao, 1977                    |
|                   | Cabbage                    | Leaf spot                               | Saudi Arabia | 1978 | Sheir <i>et al.</i> , 1981   |
|                   | <i>Brassica</i> spp.       | Leaf spot                               | U.S.A.       | 1978 | Babadoost & Gabrielson, 1979 |
|                   | seed crop                  |   |              |      | Tahvonen, 1981               |
|                   | Cabbage                    | Storage rot                             | Finland      | 1981 | Bolkan <i>et al.</i> , 1983  |
|                   | Cabbage                    | Leaf spot                               | Brazil       | 1982 | Anonymous, 1983              |
|                   | <i>Brassica</i> spp.       | Leaf spot                               | U.K.         | 1982 | Ryan <i>et al.</i> , 1984    |
|                   | <i>Brassica</i> spp.       | Leaf spot                               | Ireland      | 1983 |                              |
|                   |                            |   | Republic     |      |                              |
|                   | <i>E. sativa</i>           | Leaf blight                             | India        | 1984 | Sharma, 1985                 |
|                   | <i>Brassica</i> spp.       | Black spot                              | Spain        | 1984 | Galvez & Romero, 1988        |
|                   | oilseeds                   |   |              |      | Daebeler & Amelung, 1988     |
|                   | Rape                       | Dark leaf spot                          | Germany      | 1988 | Neergaard, 1948              |
| <b>A. raphani</b> | <i>Brassica</i> spp.       | Leaf spot                               | Denmark      | 1945 | Davis <i>et al.</i> , 1949   |
|                   | Garden                     | Leaf spot                               | California   | 1946 |                              |
|                   | Stock                      |   |              |      |                              |
|                   | Radish                     | Seed, leaf, stem, pods & root infection | USA          | 1947 | McLean, 1947                 |
|                   | <i>Matthiola</i> spp.      | Leaf spot                               | Canada       | 1953 | Connors, 1954                |
|                   | <i>B. oleracea</i>         | Leaf spot                               | Greece       | 1953 | Critopoulos, 1953            |
|                   | var. <i>capitata</i>       |   |              |      |                              |
|                   | Cabbage                    | Seed infection                          | SSSR         | 1959 | Nelen, 1959                  |
|                   | <i>Brassica</i> spp.       | Leaf spot                               | Canada       | 1963 | Taber & Vanterpool, 1963     |
|                   | Radish & <i>T. arvense</i> | Leaf spot                               | Canada       | 1966 | Petrie & Vanterpool, 1966    |
|                   | Radish                     | Leaf spot                               | Saudi Arabia | 1978 | Sheir <i>et al.</i> , 1981   |
|                   | <i>I. amara</i>            | Leaf spot                               | India        | 1981 | Narain <i>et al.</i> , 1982  |
|                   | Turnip                     | Leaf spot                               | USA          | 1982 | Cotty & Alcorn, 1984         |

#### d. Yield Losses

i) **Rapeseed - Mustard:** Heavy infections on leaves, stems, and siliquae often influence both quantity and quality of yield of Brassica crops (Butler, 1918). In India, yield losses in *Alternaria*-infected plants increase considerably after winter rains (Dey, 1948). Shrivelling of seed and reduction in quantity of oil content is the major effect in severe infections (Chahal and Kang, 1979a; Chohan, 1978; Kaushik *et al.*, 1984; Milbraith, 1922; Nijhawan and Husain, 1964; Vasudeva, 1958; Williams, 1969). The seed production of brassicas have been reported to be greatly reduced by the attack of this disease which invade siliquae and penetrate the seeds besides damaging the assimilatory tissues of the leaves and stem (Bandyopadhyay *et al.*, 1974; Nielsen, 1933). *A. brassicae* infection is also known to affect chemical composition of seed including protein, total carbohydrates and ash (Degenhardt *et al.*, 1974; Nijhawan and Husain, 1964).

In Canada Degenhardt *et al.* (1974) reported that the combined effect of *A. brassicae* and *A. raphani* infection under artificially inoculated field conditions resulted in 70 and 42% losses in yield of *B. campestris* (= *B. rapa*) and *B. napus*, respectively. According to their estimates *A. brassicae* alone can cause 63% loss in



*B. campestris* and 42% in *B. napus*; yield reductions due to *A. raphani* alone were 42% and 34%, respectively. In 1955 and 1956 crop seasons in Canada, about 20% yield losses in rapeseed have been attributed due to *A. brassicae* infection (McDonald, 1959). However, Tewari and Conn (1988) estimated an average 30% yield loss from central region of Alberta. According to Daebeler *et al.* (1986) *Alternaria* leaf spot damage in winter rapeseed ranged from 20 to more than 50% in the German Democratic Republic.

Kanwar and Khanna (1979) reported considerable deterioration in seed quality and quantity due to *Alternaria* infection. The losses in yield (Tables 2–5) have been reported from 35–45% in case of yellow sarson (Table 2; Saharan, 1984), 25–45% in brown sarson cv BSH-1 (Table 2; Chahal and Kang, 1979a), and 17–48% in raya (mustard) (Table 2; Saharan, 1984). Kolte (1982) reported 17–60% loss in yield of rai and sarson. Yield losses ranging from 10–75% have been reported in different oil-yielding crops from India (Saharan, 1992a). Yield losses are heavier in yellow sarson (38–45%) followed by brown sarson (26%) and mustard (17–18%) (Saharan, 1984, 1992a). There is a reduction in oil content from 1–10% in the infected seeds (Tables 2–5). Deep lesions on the *Brassica* siliquae increased the percentage of seed infection and decreased pod length, seeds per pod, thousand seed weight, percent seed germination and percent oil content (Tables 2–5; Bandyopadhyaya *et al.*, 1974, Chahal and Kang, 1979a; Kadian and Saharan, 1983; Nijhawan and Hussain, 1964; Kolte *et al.*, 1987; Saharan, 1984; Singh and Bhowmik, 1981; Tripathi *et al.*, 1987). Daebeler and Amelung (1988) and Saharan (1991) correlated disease intensity on foliage and/or siliquae with the components of yield losses of rapeseed-mustard. According to Ansari *et al.* (1988c), the loss in oil content of the seed from diseased plants of rapeseed cultivars over the seeds from healthy plants ranged between 14.6 and 36.0%.

Table 2. Assessment of yield losses in rapeseed-mustard due to *Alternaria* (Saharan, 1984)

| Cultivar        | Location/Year | % Disease Intensity |           | % reduction<br>in disease<br>intensity | % reduction<br>in yield | % reduction<br>in oil<br>content |
|-----------------|---------------|---------------------|-----------|--|-------------------------|----------------------------------|
|                 |               | Sprayed*            | Unsprayed |  |                         |                                  |
| Prakash (Raya)  | Hisar/79      | 18.0                | 71.1      | 74.6                                   | 17.9                    | 3.4                              |
| Varuna (Raya)   | Hisar/79      | 20.6                | 67.4      | 67.9                                   | 16.8                    | 2.5                              |
| BSH-1           | Hisar/79      | 19.7                | 62.7      | 68.5                                   | 25.6                    | -                                |
| (Brown Sarson)  |               |                     |           |  |                         |                                  |
| YSPb-24         | Hisar/79      | 19.7                | 69.9      | 71.5                                   | 35.4                    | 3.5                              |
| (Yellow Sarson) |               |                     |           |  |                         |                                  |
| RIM-514 (Raya)  | Ludhiana/81   | 41.5                | 62.5      | 33.8                                   | 48.5                    | -                                |
| YS-151          | Pantnagar/81  | 11.0                | 14.9      | 26.6                                   | 45.0                    | -                                |
| (Yellow Sarson) |               |                     |           |  |                         |                                  |
| Varuna (Raya)   | Pantnagar/81  | 9.2                 | 15.2      | 40.0                                   | 34.1                    | -                                |

\*Difolatan @ 2 g product/L water, Blitox @ 2.5 g product/L water, and Dithane M-45 @ 2 g product/L water were sprayed at Hisar, Ludhiana and Pantnagar, respectively.

Table 3. Influence of *Alternaria* pod infection on yield components of raya cultivar Prakash (Kadian and Saharan, 1983)

| Category of Infection              | Yield Components |               |                        |                   |                    |               |
|------------------------------------|------------------|---------------|------------------------|-------------------|--------------------|---------------|
|                                    | Pod length* (cm) | no. seeds/pod | no. infected seeds/pod | 1000-seed wt. (g) | % seed germination | % oil content |
| 0. Healthy pods                    | 7.2              | 19.4          | 0.0                    | 2.3               | 93                 | 40.5          |
| 1. Superficial lesions on pod      | 7.1              | 19.4          | 0.0                    | 2.3               | 93                 | 40.2          |
| 2. One to two deep lesions/pod     | 7.1              | 19.4          | 0.9                    | 2.3               | 91                 | 37.9          |
| 3. Three to five deep lesions/pod  | 6.4              | 17.4          | 2.4                    | 2.2               | 82                 | 37.7          |
| 4. More than five deep lesions/pod | 6.4              | 18.1          | 3.6                    | 2.1               | 72                 | 36.6          |
| Correlation (r)                    |                  |               |                        | 0.92              | 0.97               | 0.89          |
| * Average of 1000 pods             |                  |               |                        |                   |                    |               |

Table 4. Influence of *Alternaria* pod infection on yield components of brown sarson (Kadian and Saharan, 1983)

| Category of Infection              | Yield Components |               |                        |                   |                    |               |
|------------------------------------|------------------|---------------|------------------------|-------------------|--------------------|---------------|
|                                    | Pod length* (cm) | no. seeds/pod | no. infected seeds/pod | 1000-seed wt. (g) | % seed germination | % oil content |
| 0. Healthy pods                    | 5.9              | 18.4          | 0.0                    | 2.3               | 97.5               | 43.4          |
| 1. Superficial lesions on pod      | 4.8              | 18.2          | 0.5                    | 2.2               | 96.5               | 43.2          |
| 2. One to two deep lesions/pod     | 4.8              | 18.1          | 2.8                    | 2.1               | 85.5               | 42.5          |
| 3. Three to five deep lesions/pod  | 5.0              | 17.9          | 3.9                    | 2.0               | 81.0               | 41.3          |
| 4. More than five deep lesions/pod | 4.9              | 17.9          | 4.8                    | 2.0               | 70.0               | 38.9          |
| Correlation (r)                    |                  |               |                        | 0.98              | 0.92               | 0.87          |
| * Average of 1000 pods             |                  |               |                        |                   |                    |               |

Table 5. Influence of *Alternaria* pod infection on yield components of yellow sarson (Kadian and Saharan, 1983)

| Category of Infection              | Yield Components |               |                        |                   |                    |               |
|------------------------------------|------------------|---------------|------------------------|-------------------|--------------------|---------------|
|                                    | Pod length* (cm) | no. seeds/pod | no. infected seeds/pod | 1000-seed wt. (g) | % seed germination | % oil content |
| 0. Healthy pods                    | 7.3              | 19.3          | 0.0                    | 3.0               | 95.0               | 45.7          |
| 1. Superficial lesions on pod      | 7.3              | 19.3          | 0.0                    | 3.0               | 95.0               | 45.6          |
| 2. One to two deep lesions/pod     | 7.2              | 17.6          | 1.5                    | 2.9               | 90.0               | 44.3          |
| 3. Three to five deep lesions/pod  | 7.0              | 17.8          | 1.8                    | 2.6               | 84.0               | 43.6          |
| 4. More than five deep lesions/pod | 7.6              | 19.6          | 3.5                    | 2.2               | 69.5               | 40.3          |
| Correlation (r)                    |                  |               |                        | 0.91              | 0.86               | 0.90          |
| * Average of 1000 pods             |                  |               |                        |                   |                    |               |

Table 6. Influence of *Alternaria* infection on siliquae of *Crambe abyssinica* (Czyzewska, 1971)

| Infection categories | % seed Germination | % healthy seedlings | 1000-seed wt (g) |
|----------------------|--------------------|---------------------|------------------|
| 1. none              | 96.0               | 67.0                | 7.9              |
| 2. slight            | 95.0               | 62.5                | 7.7              |
| 3. moderate          | 94.5               | 45.5                | 6.9              |
| 4. strong            | 82.0               | 45.0                | 3.2              |
| 5. very strong       | 66.2               | 39.5                | 1.7              |

1. No infection
2. Slight infection: siliquae normal in size, several small spots on the fruit pod surface, normally developed seeds
3. Moderate infection: siliquae somewhat smaller or normal in size, numerous spots on fruit hull, seeds smaller
4. Strong infection: siliquae smaller in size, almost the whole surface of the fruit pod covered with black spots, seeds small
5. Very strong infection: siliquae deformed, the whole surface of the fruit pod covered with black spots, seeds shrunken, small, sometimes dry and black, in which case only the seed pod is left

In Europe, epidemics occur on rape about two years in every five and in these years losses may be as high as 60% in individual crops. In rape and other brassicaceous seed crops, *A. brassicae* causes yield loss due to premature ripening and shedding of seed before harvest and by reducing the 1000 grain weight. In England the disease first caused serious losses in rape in 1980-81 and since then an



estimated £3–4 million have been spent annually on fungicidal control (Smith *et al.*, 1988). In Germany *A. brassicae* caused 75% losses in rape (Klemm, 1938; Raabe, 1939).

**ii) Crambe:** In Poland in 1953 and 1954, alternaria blight of *C. abyssinica* caused very poor germination and 80% of the planting had to be ploughed or sown again (Czyżewska, 1969). Severely diseased plants produce small, wrinkled, deformed siliquae with small shrivelled and discoloured seeds having poor viability. Diseased seeds cause damping-off of seedlings leading to lower plant population in the field. The weight of 1000 siliquae can drop three times depending on the degree of infection and the weight of 1000 seeds can drop more than four times. The decrease in germination and the number of healthy seedlings can be more than 30% (Table 6) (Czyżewska, 1969, 1971; Holcomb and Newman, 1970; Leppik, 1973).

**iii) Vegetable Crops:** In vegetable crops, losses occur from damping-off of seedlings and spotting of lower leaves and heads of cabbage, turnip and rutabaga. The disease can be destructive in seedbeds, especially in cabbage, cauliflower and brussels sprouts. Spotting and browning of cauliflower, broccoli and cabbage heads reduce quality and market value of these crops (Sherf and Macnab, 1986). *A. brassicicola* is most important on *B. oleracea* seed crops. Reduction in seed yield may be as high as 80% and the fungus may severely depress germination to the extent that infected seed may be unsaleable (Smith *et al.*, 1988). *A. brassicicola* has recently been recognized as an important cause of deterioration of white cabbage in cold storage (Kear *et al.*, 1977). According to Gorshkov (1976), damping-off of cabbage caused by *A. brassicae* may result into 80–100 percent losses. In wet seasons in the USA, *A. brassicae* leaf spot on cabbage, cauliflower, broccoli and other brassicas has been known to reduce the yield by more than 50%. The market losses are due to decay which develops in transit and storage (Ramsey and Smith, 1961). In Magdeburg district of Germany, *Alternaria* caused seed losses of up to 50% in cauliflower seed plants (Stoll, 1948), and from 70 to 90% of the harvest on rape and seed cabbage plantings (Domsch, 1957).

*Alternaria alternata* infection in radish reduces pod length, number of seeds per pod and thousand seed weight leading to reduction in seed yield by as much as 18% (Suhag *et al.*, 1983).

#### **e. Disease Assessment Keys/Severity Charts**

*Alternaria* diseases can be assessed by various methods described below:

**i) Visual assessment methods:** There are two well documented visual assessment methods.

**(a) Descriptive keys:** In diagrams, the plants with varying amounts or types of disease symptoms are categorized with an accompanying description. The descriptive keys for assessment of disease severity used by different workers are given in Table 7. These keys measure the disease in a scoring scale of 0–5 or 0–9. The zero (0) score indicates no symptoms on any part of the host, and 1–5 or 1–9 scores indicate the presence of different degrees of disease symptoms on various

host parts. A score of 1 indicates the least amount of disease and scores of 5 or 9 indicate maximum disease (75 % or more) of the host infected. These descriptive keys are successfully used for estimating disease severity of host plants of differing disease resistance or of host plants subjected to differing environmental conditions or cultural procedures. Host resistance is measured by categorization based on the system described above: resistant (0–1), moderately resistant (1.1–3), moderately susceptible (3.1–5), susceptible (5.1–7), and highly susceptible (7.1–9). Quantitative estimates of the disease are made in this form of disease index using numerical rating based on the severity of the disease (McKinney, 1923):

$$\text{Disease Index (\%)} = \frac{\sum (\text{sample frequency} \times \text{numerical rating } 1) + \dots + \text{sample frequency} \times \text{numerical rating } 5 \text{ or } 9}{\text{Total no. samples} \times \text{maximum numerical rating } 5 \text{ or } 9} \times 100$$

Whenever disease assessments are recorded, the growth stage of the crop should be noted so that the disease progress can be related to host development. Growth stage key of rapeseed is given in Table 8.

An improved grading system for measuring plant diseases was proposed by Horsfall and Barratt (1945) which has been used by Fontem *et al.* (1991) to measure the progress and spread of dark leaf spot in cabbage. This grading is based on the principle that according to the Weber-Fechner law, the human eye distinguishes according to the logarithm of the light intensity. Hence, the grades should be based on the ability to distinguish, rather than the extent of the disease. Below 50%, the eye sees the amount of diseased tissue. Above 50%, it sees the amount of diseased free tissue. This scoring system (1–12) is based on 50% as a mid-point. The grades differ by a factor of two in either direction (Table 7). Several plants (20 or more) at random are graded.

$$\text{Mean grade} = \frac{\text{grade reading}}{\text{number of reading}}$$

A calibration curve is set up with grade numbers on the x - axis and percentage disease on a special semi-log. Y-axis with one and one-half phases from either end up to 50%. This scheme has been very useful in testing the efficacy of fungicides, varietal resistance and in surveys of plant disease. Fontem *et al.* (1991) used the conversion table of Redman *et al.* (1967) to convert the Horsfall-Barratt rating score to disease proportions in black spot of cabbage.

**(b) Standard area diagrams:** Pictorial representation of the host plant with known and graded amounts of disease are compared with diseased leaves and / or siliquae to allow estimation of disease severity. It consists of a set of pictures giving a schematised illustration of the grades distinguished as in Figure 3 (Conn *et al.*, 1990).

In contrast to descriptive keys, standard area diagrams allow estimation of intermediate levels of disease severity by comparing a diseased plant with diagrams showing both more and less disease. To calculate the disease severity, the leaf and/or siliquae to be assessed are matched to one of the diagrams of the black areas,



or area damaged, shown (representing 1%, 5%, 10%, 20%, 30%, and 50%) for each leaf and/or siliquae covered by the actual lesions. Disease severity is calculated using the following formula.

$$\text{Disease severity (Area) \%} = \frac{\text{Area of the plant tissue affected by disease}}{\text{Total area}} \times 100$$

Although only a few representative percentage infections (1, 5, 10, 20, 30, 50) are given in the diagrams for assessment of severity, interpolations for arriving at the middle level percentage such as 2, 3, 15, 30, 40, 60 etc. can be easily practised and recorded. The extent of interpolation will be dictated by the ability of the observer to detect particular differences. Once the severity percentages are decided upon, the visual scales/keys can be used for categorization. However, once the observer becomes familiar with the diagrams, the ranking numbers can be used as such for comparison between the genotypes on 0–5 or 0–9 scale. It is expected that 10 percent of the representative population be considered for assessment. The percentage severity data as such can be transformed for any subsequent epidemiological analysis by using the disease index formula given earlier.

The standard area diagrams constructed recently (James, 1974) account for the logarithmic decrease in the activity of the eye in estimating disease severities approaching 50% in their selection of representative keys. Estimations of disease severity intermediate between two keys are often made by careful interpolation. As logistic transformation fits many disease progress curves, equal interval grades on the logit scale are being often used (James, 1974).

**ii) Incidence - Severity Relationships:** The relationship between incidence and severity (I–S relationship) is an epidemiologically significant concept. Since incidences are easier to measure than severity, any quantifiable relationship between the two measures permit estimation of severity based upon incidence data, which are more precise and easily acquired. Where resource limitations prevent the collection of accurate severity measurements, estimation of severity based upon incidence data will be highly beneficial in disease and yield loss assessments. The I–S relationship can be used through the analysis of correlation and regression, multiple infection methods and measurement of aggregation (Seem, 1984). There is enormous scope in exploring the I–S relationship especially in understanding the type of disease spread in a sampling unit in *Alternaria* diseases.

**iii) Inoculum - Disease Intensity Relationships:** An alternative method of disease assessment, particularly suitable in studies of host resistance is one based on the count of number of spores produced per lesion. While analyzing components of horizontal resistance in rapeseed-mustard cultivars against *A. brassicae*, Saharan and Kadian (1983), indicated spore count per lesion as one of the most important parameters. An optimum inoculum concentration must be determined to obtain differences in susceptibility among cultivars/genotypes (Dueck and Degenhardt, 1975).

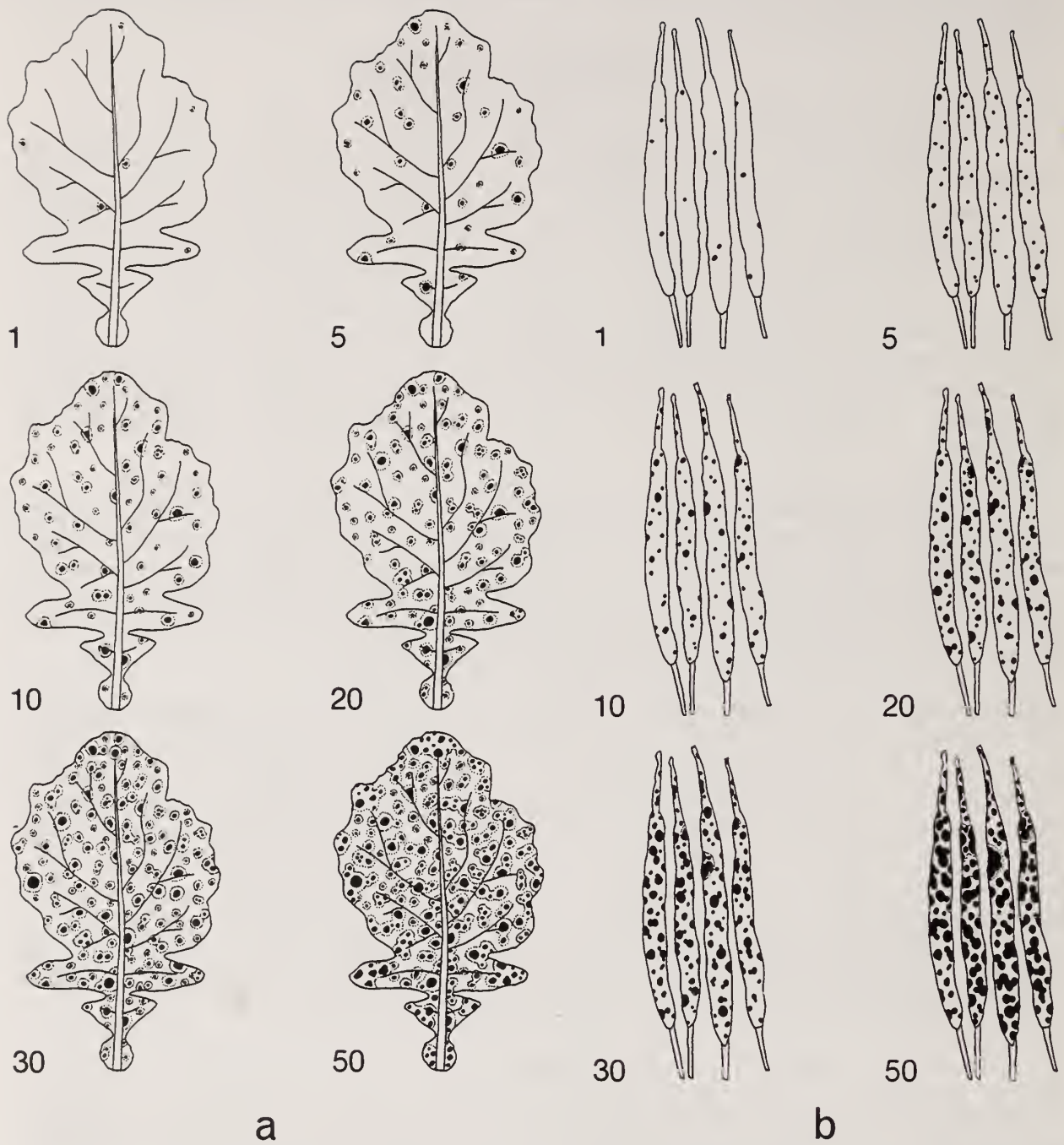


Fig. 3. A schematized drawings of (a) leaves; and (b) siliquae of crucifers showing *Alternaria* infection grades (Courtesy: Conn, Tewari and Awasthi, 1990).

**iv) Remote Sensing Method:** Aerial infra-red photography using remote sensing procedure is commonly utilized to detect plant diseases. The detection of diseased plant tissue on false-colour infrared film is due to its greater reflection of near infrared light (=700–950 nm) compared to healthy tissue. Colour infrared photographs have been analysed with microdensitometers or other types of electronic scanning devices to quantify the disease severity. This technique is useful to employ in areas where the same crop is contiguous over large areas. Use of this technique in assessing incidence of *Alternaria* diseases has not yet been reported.

**v) Video Image Analysis:** This method can be easily employed for assessing *Alternaria* diseases of brassicaceous plants since its lesions and healthy tissue have different colours. Recent advances in electronic and computer technology allow video cameras to interface directly with a microcomputer. Rapid, automated, nonsubjective estimates of disease severity are made possible by computer-controlled analysis of video images. The accuracy of estimates of disease severity, obtained using video analysis is high and appears to be independent of both the complexity of the host-pathogen system and disease severity.

Table 7. Keys for the assessment of *Alternaria* disease severity on crucifers (Mayee and Datar, 1986)

| Scoring Scale | Description  |
|---------------|--|
| 0.            | No symptoms on leaf  |
| 1.            | Small, round, black dots covering 1 % or less of the leaf area   |
| 3.            | Gray, circular spots containing concentric rings, to cover 1 - 10% of leaf area                                  |
| 5.            | Lesions enlarge, gray circular spots with concentric rings and a black border to cover 11 - 25% of the leaf area |
| 7.            | Lesions enlarge and coalesce with each other to cover 26 - 50% of the leaf area                                  |
| 9.            | Lesions coalesced, with concentric rings and black border to cover 50% or more of leaf area, defoliation occurs  |

Saharan, 1991

|    |                                     |
|----|-------------------------------------|
| 0. | Healthy pods                        |
| 1. | Minute superficial lesions per pod  |
| 2. | One to two deep lesions per pod     |
| 3. | Three to five deep lesions per pod  |
| 4. | Five to eight deep lesions per pod  |
| 5. | More than nine deep lesions per pod |



Horsfall and Barratt (1945); Fontem *et al.* (1991)

|     |                         | <u>Values to convert back to %</u> |                   |
|-----|-------------------------|------------------------------------|-------------------|
|     |                         | Mid Point                          | Elanco Formula %* |
| 1.  | No symptoms             | 0                                  | 0                 |
| 2.  | 0 - 3% area diseased    | 1.50                               | 2.34              |
| 3.  | 3 - 6% area diseased    | 4.50                               | 4.68              |
| 4.  | 6 - 12% area diseased   | 9.00                               | 9.37              |
| 5.  | 12 - 25% area diseased  | 18.50                              | 18.75             |
| 6.  | 25 - 50% area diseased  | 37.50                              | 37.50             |
| 7.  | 50 - 75% area diseased  | 62.50                              | 62.50             |
| 8.  | 75 - 87% area diseased  | 81.50                              | 81.25             |
| 9.  | 87 - 94% area diseased  | 91.00                              | 90.63             |
| 10. | 94 - 97% area diseased  | 96.50                              | 96.31             |
| 11. | 97 - 100% area diseased | 98.50                              | 97.66             |
| 12. | 100% area diseased      | 100                                | 100               |

\*Redman *et al.*, 1967

Braverman, 1971

|     |                                  |
|-----|----------------------------------|
| 0   | Zero percent leaf area affected  |
| 0.5 | Traces to 10% leaf area affected |
| 1.0 | 11 - 20% leaf area affected      |
| 1.5 | 21 - 30% leaf area affected      |
| 2.0 | 31 - 40% leaf area affected      |
| 2.5 | 41 - 50% leaf area affected      |
| 3.0 | 51 - 60% leaf area affected      |
| 3.5 | 61 - 70% leaf area affected      |
| 4.0 | 71 - 80% leaf area affected      |
| 4.5 | 81 - 90% leaf area affected      |
| 5.0 | 91 - 100% leaf area affected     |

### 3. PATHOGEN

#### a. Historical

As early as 1836, Berkeley identified the causal fungus on plants belonging to the Brassicaceae as *Macrosporium brassicae* Berk., which was later renamed as *A. brassicae* (Berk.) Sacc. by Saccardo (1886). In 1922, 1926 and 1945, respectively, Milbraith, Weimer and Rangel also described the same fungus. Then in 1947, Wiltshire separated the small and big spores forms as *A. brassicicola* (Sch.) Wiltshire and *A. brassicae* (Berk.) Sacc., respectively.

Every modern attempt to define the phaeodictyosporic form genera *Alternaria* Nees ex Fries and *Stemphylium* Wallroth, has involved the problem of

treating numerous taxa which superficially resemble the type species of one or the other of these genera, but which are sufficiently different as to leave a measure of doubt. Chief among these anomalous species are *Stemphylium lanuginosum* Harz and *Macrosporium consortiale* Thiimen, both of which, at one time or another, have been named *Alternaria*, *Stemphylium*, or *Pseudostemphylium* (Simmons, 1967).

Wiltshire (1933, 1938) pioneered the basic studies of this group of Hyphomycetes and published the results of his examination of the available type specimens. His descriptive literature was fundamental to the prevailing concepts of *Alternaria*, *Macrosporium* and *Stemphylium*. His major conclusions were that *Macrosporium* should be suppressed as a *nomen ambiguum* in favor of *Alternaria*, typified by *A. tenuis* Nees, the type specimen of which Wiltshire was unable to locate for examination, and that the limits of *Stemphylium* should be modified to include two sections.

Neergaard (1945), in his extensive treatment of species of *Alternaria* and *Stemphylium* occurring in Denmark, recognized the same taxonomic problems in handling species similar to *S. lanuginosum*. He also followed the lead of Wiltshire by retaining the two sections proposed for *Stemphylium*.

Joly (1964) in his survey of *Alternaria* differentiated *Stemphylium* in its original sense (and in the sense of Wiltshire's section *Eustemphylium*) and transferred to *Alternaria* several of the taxa similar to *S. lanuginosum* which were considered controversial by earlier students of the group. Most taxonomists recognized that *Alternaria* and *Stemphylium* were inappropriate generic designations for species similar to *S. lanuginosum*. By their nomenclatural proposals or, more importantly, by their invariably excellent illustrations, conidiophores of members of the *S. lanuginosum* group bear no resemblance whatsoever to those of *S. botryosum* and the conidial morphology of the group is fundamentally different from that of the type species of *Alternaria*.

There are two species of *Alternaria* found on species of *Brassica* which are probably more confused than any other species in this genus (Wiltshire, 1947). The first is *A. brassicae* (Berk.) Sacc. described as *Macrosporium brassicae* Berk. in 1836 and the second is the fungus commonly known as *A. circinans* (Berk. & Curt.) Bolle. or *A. oleracea* Milbraith or, incorrectly as *A. brassicae* (Berk.) Sacc. for which Wiltshire (1947) proposed the name *A. brassicicola* (Schwein.).

## **b. Taxonomy and Morphology**

*Alternaria nees* ex. Fr., Nees, Syst. Pilze Schw.:72; 1816; Fries, Syst. mycol. 1:XLVI, 1821  
= *Macrosporium* Fr., 1832  
= *Rhopalidium* Mont., 1846

Colonies effuse, usually grey, dark blackish brown or black. Mycelium immersed or partly superficial; hyphae colourless, olivaceous brown or brown. Stroma rarely formed. Setae and hyphopodia absent. Conidiophores macronematous, simple or irregularly and loosely branched, pale brown or brown, solitary or in

fascicles. Conidiogenous cells terminal becoming intercalary, polytretic, sympodial, or sometimes monotretic. Conidia catenate or solitary, dry, typically ovoid or obclavate, often rostrate, pale or mild-olivaceous brown or brown, smooth or verrucose, with transverse and frequently also oblique or longitudinal septa (Ellis, 1971; Holliday, 1980).

The following species of this genus cause economically important diseases in Brassicaceae.

***Alternaria alternata*** (Fr.) Keissler, Beih. Bot. Zbl., 29:434, 1912  
= *Torula alternata* Fr., 1832, Syst. Mycol., 3:500.  
= *A. tenuis* C.G. Nees, 1916/17, Syst. Pilze Schwamme:72.

The reasons why the epithet *alternata* should be used instead of the more commonly accepted one *tenuis* are clearly stated by Simmon (1967).

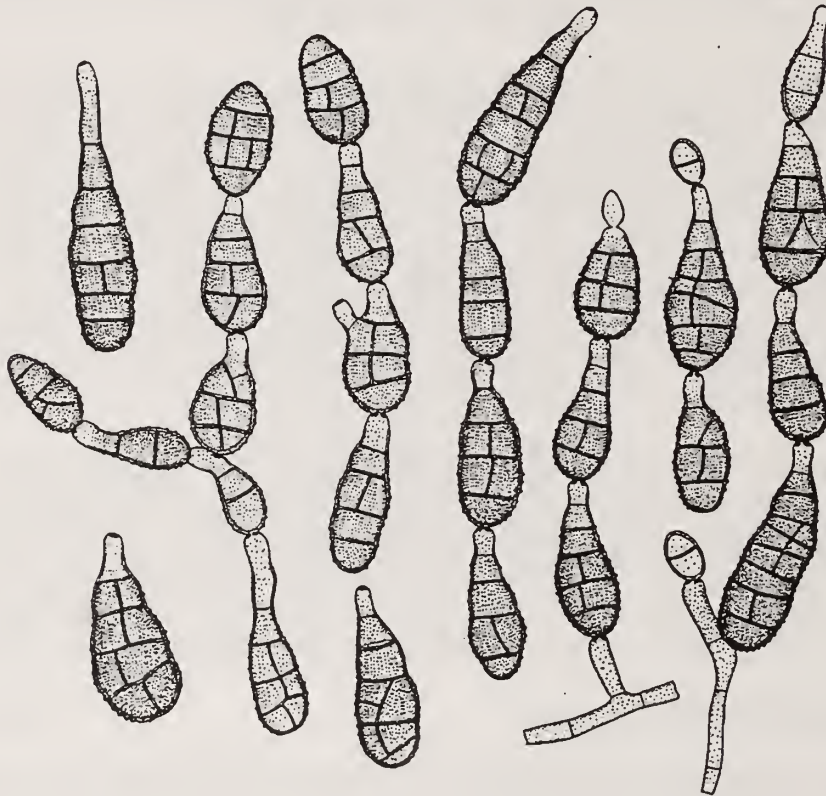


Fig. 4. *Alternaria alternata* (x 650) (Courtesy: Ellis, 1971).



Table 8. Growth stage key for oilseed rape (NIAB, 1985)

| GROWTH STAGE               | DECIMAL CODE | CHARACTERISTICS                       |
|----------------------------|--------------|---------------------------------------|
| Germination                | 0.0          | Dry seed                              |
| Emergence                  | 0.8          | Cotyledons emerged                    |
| Leaf production            | 1.00         | Cotyledons unfolded                   |
|                            | 1.01         | First true leaf emerged               |
|                            | 1.02 etc.    | Second true leaf emerged              |
| Stem extension             | 2.00         | No internodes (rosette)               |
|                            | 2.01         | One internode                         |
|                            | 2.02 etc.    | Two internodes                        |
| Flower and bud development | 3.0          | Leaf buds only                        |
|                            | 3.3          | Green flower buds visible             |
|                            | 3.7          | Yellow flower buds visible            |
| Flowering                  | 4.1          | First flower opened                   |
|                            | 4.2          | 20% buds on terminal racemes flowered |
|                            | 4.3 etc.     | 30 % buds flowered                    |
| Pod development            | 5.1          | Lowest pods 2 cm long                 |
|                            | 5.2          | 20 % pods 2 cm long                   |
|                            | 5.3 etc.     | 30 % pods 2 cm long                   |
| Seed development           | 6.1          | Seeds present                         |
|                            | 6.2          | Seeds green                           |
|                            | 6.5          | Seeds brown                           |
|                            | 6.7          | Seeds black but soft                  |
|                            | 6.9          | All seeds black and hard              |

Colonies usually black or olivaceous black, sometimes grey. Conidiophores arising singly or in small groups, simple or branched, straight or flexuous, sometimes geniculate, pale to mild olivaceous or golden brown, smooth, up to 50  $\mu$  long, 3–6  $\mu$  thick with 1 or several conidial scars. Conidia formed in long, often branched chains, obclavate, obyriform, ovoid, or ellipsoidal, often with a short conical or cylindrical beak, sometimes up to but not more than one-third the length of the conidium, pale to mid golden brown, smooth or verruculose with up to 8 transverse and usually several longitudinal or oblique septa, overall length 20–63 (37)  $\mu$ , 9–18 (13)  $\mu$ , thick in the broadest part; beak pale, 2–5  $\mu$  thick (Fig. 4). An extremely common saprophyte found on many kinds of plants and other substrata including foodstuff, soil and textiles; cosmopolitan (Ellis, 1971; Holliday, 1980).

- Alternaria brassicicola*** (Schw.) Wiltshire in Mycol. Pap. 20:8, 1947  
= *Helminthosporium brassicicola* Schweinitz (as *Helminthosporium brassicola*) in Trans. AM. Phil. Soc. N.S., 4 : 279, 1832  
= *Macrosporium cheiranthi* Fr. var. *circinans* Berk. & Curt. in Grevilles, 3: 105, 1875  
= *Alternaria circinans* (Berk. & Curt.) Bolle in Meded. Phytopath. Lab. Willie commelin Scholten, 7:26, 1924  
= *Alternaria oleracea* Milbraith in Bot. Gaz., 74: 320, 1922  
(Full synonymy given by Wiltshire in Mycol. Pap. 20: 1947)

Colonies amphigenous, effused, dark olivaceous brown to dark blackish brown, velvety. Mycelium immersed; hyphae branched septate, hyaline at first, later brown or olivaceous brown, inter- and intra-cellular, smooth, 1.5–7.5  $\mu$  thick. Conidiophores arising singly or in groups of 2–12 or more, emerging through stomata, usually simple, erect or ascending, straight or curved, occasionally geniculate, more or less cylindrical but often slightly swollen at the base, septate, pale to mild olivaceous brown, smooth up to 70  $\mu$  long, 5–8  $\mu$  thick. Conidia mostly in chains of up to 20 or more, sometimes branched, acropleurogenous, arising through small pores in the conidiophore wall, straight, nearly cylindrical, usually tapering slightly towards the apex or obclavate, the basal cell rounded, the beak usually almost non-existent, the apical cell being more or less rectangular or resembling a truncated cone, occasionally better developed but then always short and thick, with 1–11, mostly less than 6, transverse septa, often constricted at the septa, pale to dark olivaceous brown, smooth or becoming slightly warted with age, 18–130  $\mu$  long, 8–30  $\mu$  thick in the broadest part, with the beak 1/6 the length of the conidium and 6–8  $\mu$  thick (Ellis, 1968b, 1971; Holliday, 1980). The fungus is confined to the Brassicaceae (Fig. 5).

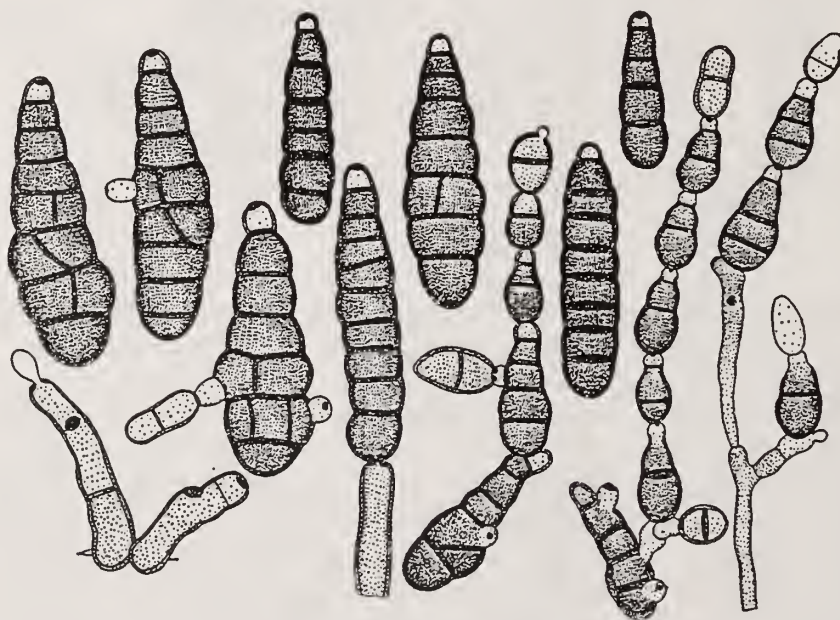


Fig. 5. *Alternaria brassicicola* (x 650) (Courtesy: Ellis, 1971).



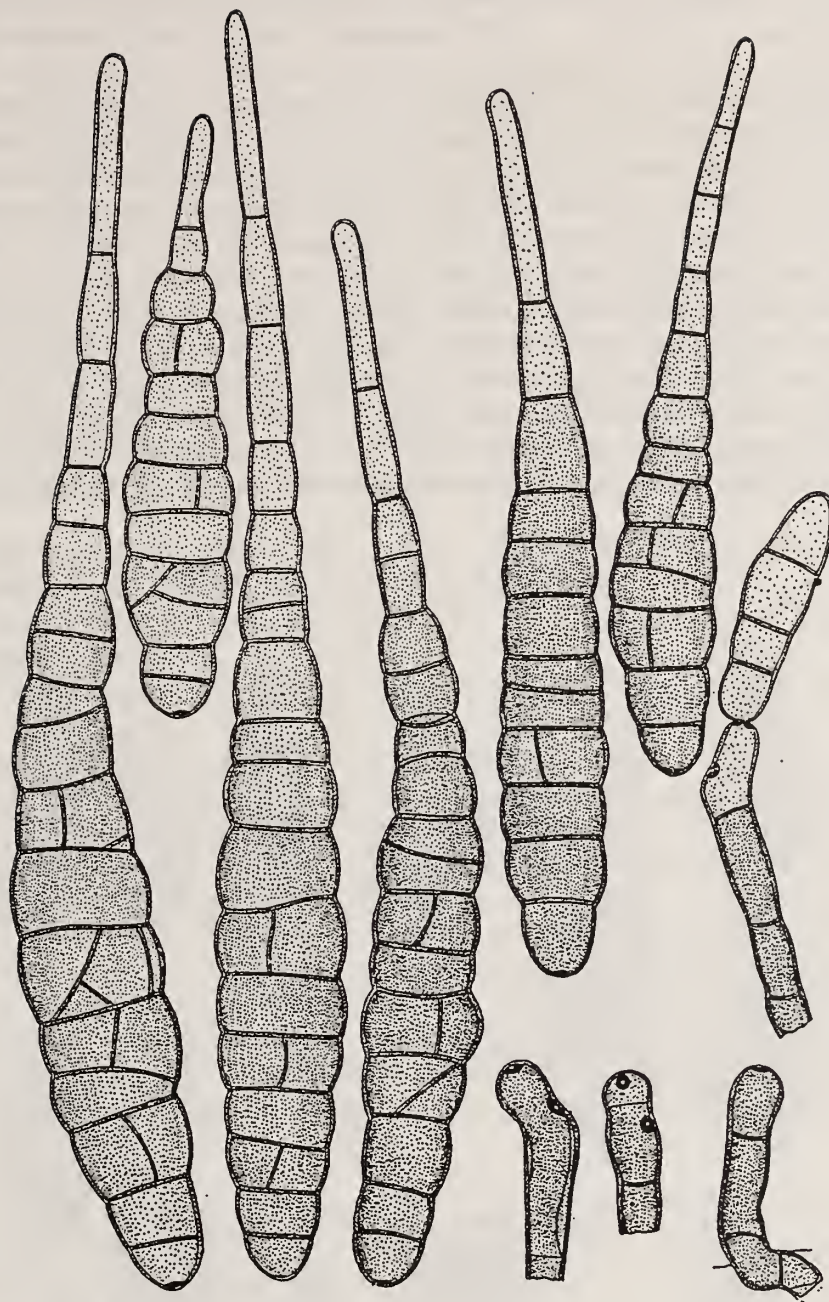


Fig. 6. *Alternaria brassicae* (x 650) (Courtesy: Ellis, 1971).

***Alternaria brassicae*** (Berk.) Sacc. in Michelia, 2:129 see also p. 172, 1880  
= *Macrosporium brassicae* Berk. in Smith's Engl. Fl. 5, pt. 2 : 339, 1836  
(Full synonymy given by Wiltshire in Mycol. Pap., 20, 1947).

Colonies amphigenous, effused, rather pale olive, hairy, the individual large conidia plainly seen under a x 20 binocular dissecting microscope. Mycelium immersed; hyphae branched, septate, hyaline, smooth, 4–8  $\mu$  thick. Conidiophores arising in groups of 2–10 or more from the hyphae, emerging through stomata, usually simple, erect or ascending, straight or flexuous, frequently geniculate, more or less cylindrical but often slightly swollen at the base, septate, mid-pale greyish olive smooth up to 170  $\mu$  long, 6–11  $\mu$  thick, bearing one to several small but distinct conidial scars. Conidia solitary or occasionally in chains of up to 4, acropleurogenous, arising through small pores in the conidiophore wall, straight or slightly curved, obclavate, rostrate with 16–19 (usually 11–15) transverse septa and 0–8 (usually 0–3) longitudinal or oblique septa, pale or very pale olive or greyish olive; smooth or infrequently, very inconspicuously warted, 75–350  $\mu$  long and usually 20–30  $\mu$  (sometimes up to 40  $\mu$ ) thick in the broadest part, the beak about 1/3 to 1/2 the length of the conidium and 5–9  $\mu$  thick (Fig. 6) (Ellis, 1968a, 1971; Holliday, 1980). This species produces chlamydospores. It is mostly confined to the Brassicaceae.

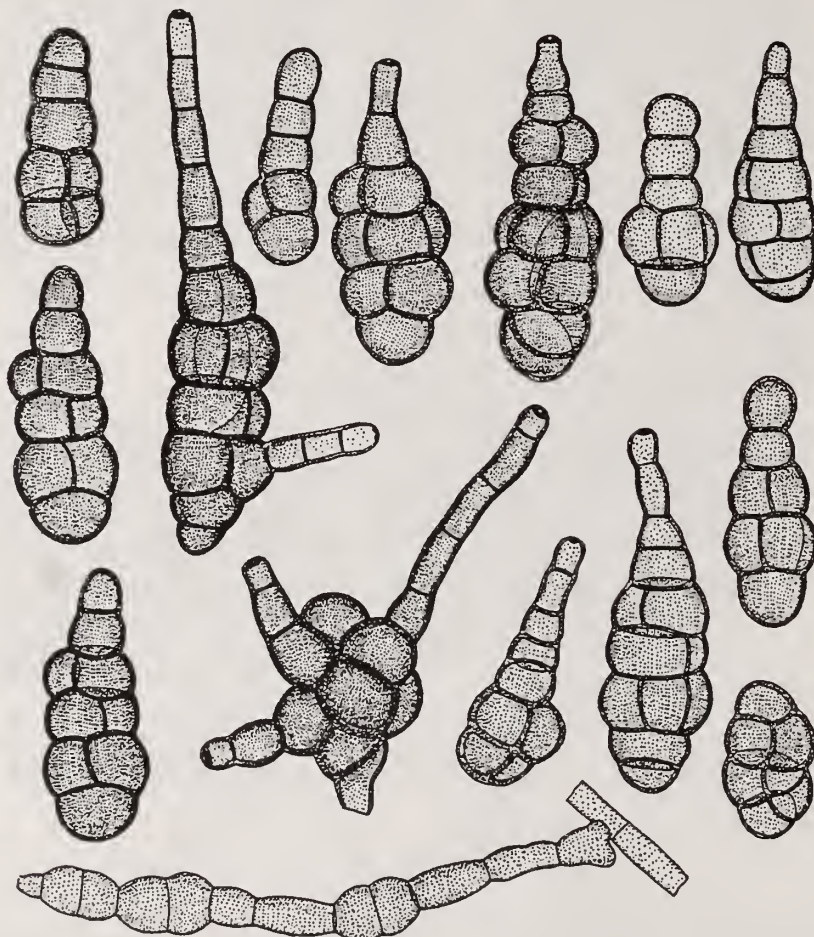


Fig. 7. *Alternaria raphani* (x 650) (Courtesy: Ellis, 1971).



***Alternaria raphani*** Groves & Skolko, 1944, Can. J. Res., Sect. C, 22:227.  
= *Alternaria matthiolae* Neergaard, 1945

Conidiophores simple or occasionally branched, septate, olivaceous brown, up to 150  $\mu$  long, 3–7  $\mu$  thick, sometimes swollen slightly at the tip and usually with a single conidial scar, conidia commonly in chains of 2–3, straight or slightly curved, obclavate or ellipsoidal, generally with a short beak, mid to dark golden brown or olivaceous brown, smooth or sometimes minutely verruculose, with 3–7 transverse and often a number of longitudinal or oblique septa; constricted at the septa, 50–130 (70)  $\mu$  long, 14–30 (22)  $\mu$  thick in the broadest part. Chlamydospores formed abundantly in culture, sometimes in chains at first 1 celled, round, finally many-celled and irregular, brown; Conidiophores often develop from them. (Ellis, 1971; Holliday, 1980). Unlike *A. alternata* and *A. brassicicola*, this species forms chlamydospores and the beak of the conidium is smaller than that of *A. brassicae* but longer than the almost nonexistent beak of *A. brassicicola* (Fig. 7).

***Alternaria cheiranthi*** (Lib. Bolle, 1924, as "(Fr.) Bolle", Meded. phytopath. Lab. Willie Commelin Scholten, 7:55.  
= *Helminthosporium cheiranthi* Lib., 1827, apud Desm., Crypt. Fr. Exsicc., 213.  
= *Macrosporium cheiranthi* (Lib.) Fr., 1832, Syst. mycol., 3: 374.

Conidiophores arising singly or in groups, mostly simple but sometimes branched, straight or flexuous, septate, rather pale olive, often hyaline at the apex, smooth, up to 130  $\mu$  long, 5–8  $\mu$  thick with a single terminal scar at first but later with up to 4 scars which may be borne close together without marked geniculation. Conidia mostly solitary, rarely in chains of 2, 3 or more, variously shaped, often pyriform, ovoid or elongate ovoid at first, later becoming irregular, mostly tapering to the apex which may be drawn out into a beak, generally rounded at the base, with numerous transverse, longitudinal and oblique septa, light olive to golden brown, translucent, with the interior walls, which are dark, often plainly visible, smooth or with the wall pitted from the inside, 20–100  $\mu$  long, 13–32  $\mu$  thick in the broadest part (Fig. 8). Common on wallflowers and occasionally on other brassicaeous plants (Ellis, 1971).

To establish the correct systematic designation of two common species of *Alternaria* on Brassicaceae Wiltshire (1947) gave the following account of synonymy which had appeared in the literature up to 1945.

1. ***Alternaria brassicae*** (Berk.) Sacc.

- 1836. *Macrosporium brassicae* Berk. In Smith's Engl. Flora. v, Part II P. 339 (=Hooker's British Flora, vol.ii, part II, 'Fungi' by M.J. Berkeley.
- 1836. *Puccinia* (?) *brassicae* Montagne, In Ann. Sci. Nat., Ser. II VI, p. 30.
- 1855. *Sporidesmium exitiosum* Kühn In Hedwigia, i p. 91
- 1856. *Rhopalidium brassicae* Mont. & Fr. In Montagne's Syll. Crypt., P.297.
- 1859. *Polydesmus exitiosus* (Kühn) Kühn. In Kramkh. d. Kulturgew., P.165.
- 1880. *Alternaria brassicae* (Berk.) Sacc. var. minor Sacc. In Mich. ii, P. 172.
- 1882. *Cercospora bloxami* Berk. & Br. In Ann. & Mag. Nat. Hist. Ser. V, IX. P. 183, No. 1882, 1879.

1882. *Macrosporium herculeum* Ellis & Martin, In Amer. Nat., XVI, p.1003; exsicc. N. Amer. Fungi, 1263.
1884. *Cercospora lepidii* Peck. In 35th Rept. N.Y. State Mus. 1881, p. 140.
1886. *Alternaria brassicae* (Berk.) Sacc. var. *macrospora* Sacc. In Syll., IV, p. 546.
1891. *Sporidesmium annii* Karst. In Symb. Myc.Fenn. XXX, P. 67.
1897. *Macrosporium brassicae* Berk. var. *macrospora* Eliason.
1901. *Sporidesmium brassicae* Massee In Kew. Bull., 1901, P. 153.
1902. *Leptosphaeria exitiosa* (Kühn) Rostrup In Plantepatologi Haandbog i Laeren om Plantesygdomme for Landbrugere, Havebrugere og Skovbrugere, P. 472.
1902. *Alternaria brassicae* (Berk.) Sacc. var. *exitiosa* (Kühn) Ferraris. In Flora italica, P. 521.
1917. *Alternaria herculea* (Ell.& Mart.) Elliott. In Amer. Journ. Bot., IV, p. 472.
1924. *Alternaria brassicae* (Berk.) Bolle [nee. Sacc.]
1944. *Alternaria macrospora* (Sacc.) Sawada. In Descriptive catalogue of the Formosan fungi- Part V, Dept. Agric. Govt. Res. Inst. Formosa, Rept. 51, p. 123.
1945. *Alternaria exitiosa* (Kühn) Jorstad. In Melding fra Statens. Plantepatogiske Institut. No. 1, P. 594.

It is extremely confusing that the name *A. brassicae* (Berk.) Sacc. has been applied, in accordance with Saccardo's usage in the Sylloge, to the species *A. brassicicola* by various authors, e.g. Voglino (1902), Ferraris (1912), Sawada (1931), Weber (1932), Yoshii (1933), Fajardo and Palo (1934).

## 2. *Alternaria brassicicola* (Schwein) Wiltshire.

1832. *Helminthosporium brassicicola*. Schweinitz In Syn. Fung. Amer. Bar. No. 2632, Trans. Amer. Phil. Soc., N.S., IV P. 279.
1855. *Sporidesmium exitiosum* Kühn formae (B) *Alternarioides* and (Y) *luxuriosum* Kühn In Hedwigia, I, P. 91.
1859. *Polydesmum exitiosus* (Kühn) Kühn formae (B) *Alternarioides* and (Y) *luxuriosum* (Kühn) Kühn In Krankh. d. Kulturgew., p. 165.
1875. *Macrosporium cheiranthi* Fr. var. *circinans* Berk. & Curt. In Grevillea, III, P. 105.
1880. *Alternaria brassicae* (Berk.) Sacc. *minor* Sacc. In Mich., II, P. 172.
1886. *Macrosporium commune* Rabenh. var. *circinans* (Berk. & Curt.) Sacc. In Syll., iv, P. 524.
1886. *Alternaria brassicae* (Berk.) Sacc. In Syll., IV, p. 546 .
1897. *Alternaria brassicae* (Berk.) Sacc. var. *microspora* Brun, In Act. Soc. linn. Bordeaux, III, P. 149.
1902. *Helminthosporium brassicae* P. Henn. In Hedwigia, XLI, p. 117.
1922. *Alternaria oleracea* Milbraith In Bot. Gaz., LXXIV, p. 320.
1924. *Alternaria circinans* (Berk. & Curt.) Bolle In Meded. Phytopath. Lab. Willie Commelin Scholten; Baarn, VII, P. 26.
1933. *A. brassicae* (Berk.) 'Lindau' cited in error by Yoshii for *A. brassicae* (Berk.) Sacc.



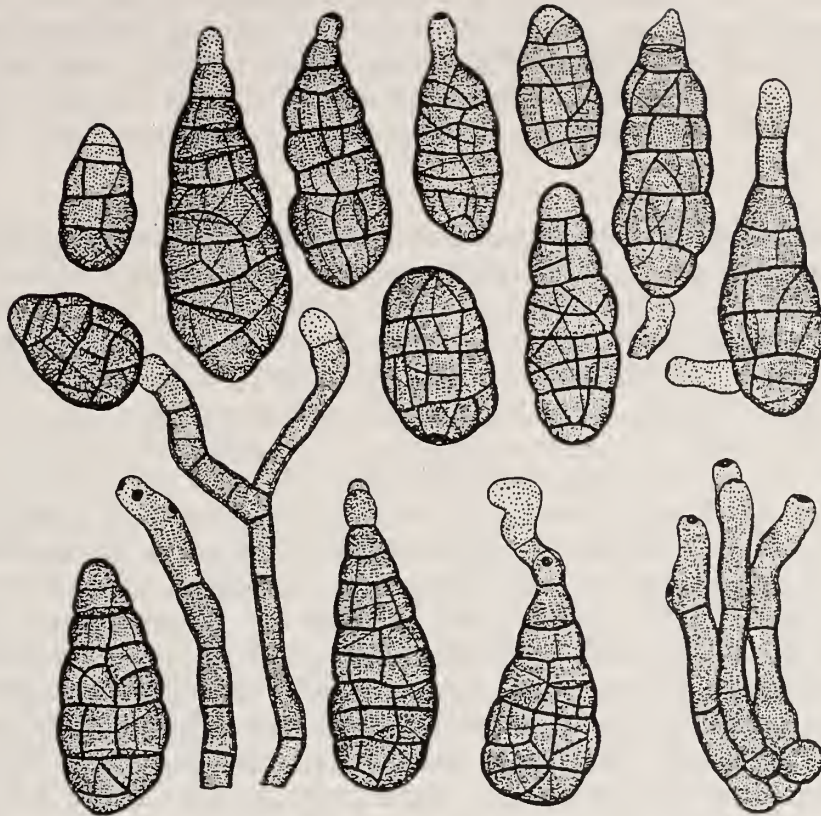


Fig. 8. *Alternaria cheiranthi* (x 650) (Courtesy: Ellis, 1971).

Reviewing then the names that have been applied to *A. brassicicola* it is evident that Schweinitz's name predates the others by many years. There is no doubt that *A. oleracea* and *A. circinans* are identical with it and must rank as synonyms. The species is quite distinct from the true *A. brassicae* (Berk.) Sacc. and though *A. brassicicola* shows considerable variations on the host, in culture it always produces the same characteristic, almost cylindrical, short beaked spores of 11.5–44 by 7–9  $\mu$  by which its identity can readily be established (Wiltshire, 1947).

### c. Physiological Specialization and Variability

Variations in cultural characteristics and pathogenesis of different isolates of four *Alternaria* species infecting Brassicaceae have been observed. However, information on existence of distinct pathotypes using standard host differentials is rather limited (Saharan, 1992b).

In *A. alternata* strains showing differences in their physiological and pathological characteristics have been isolated from Crambe (*C. abyssinica*). Strain A occurs on leaves, stem and siliquae whereas strains B and C are mainly found on

siliquae and leaves, respectively. In pathogenic ability, strain B has been reported to be most virulent, strain A as moderately virulent, and strain C as least pathogenic (Czyzewska, 1969, 1971). These strains have different temperature optima for sporulation. Strain A sporulates abundantly at 17–35°C whereas strain B requires 20–30°C temperature and strain C will sporulate best at 12°C (Czyzewska, 1970).

*Alternaria brassicae* is considered to be most virulent on all brassicaceous plants. Preliminary reports on variability in this species were made from Holland (Van Schreven, 1953) and UK (Mridha, 1983). Isolates of *A. brassicae* obtained from rapeseed (colza) showed differences in cultural growth on cherry agar and differed in their pathogenesis on seedlings of colza. Similarly, Kolte *et al.* (1989, 1991) and Awasthi and Kolte (1989) distinguished three isolates viz., A, C and D of *A. brassicae* on the basis of their morphology, sporulation, growth and cultural characteristics. On *B. carinata* these isolates produce distinct types of lesions. Among the three isolates, isolate C sporulates the most and isolate A the least. Unlike isolates B and C, isolate A produces chlamydospores. In a serological study, Kolte *et al.* (1991) indicated that the Pantnagar isolates A, C, and D resembled the Bihar isolates BH1, BH2 and Kanpur isolates K, respectively. None of these workers used different *Brassica* species to distinguish isolates of *A. brassicae* on the basis of their virulence. Saharan and Kadian (1983) used eight commonly cultivated *Brassica* species to distinguish isolates of *A. brassicae* infecting these crops in India. In a cross-infection study they could distinguish between three isolates showing differential interaction. These isolates were designated as RM1, RM2 and V3. All the three races in this study were found to be virulent on rapeseed and mustard group of crops. Race RM1 was isolated from rapeseed-mustard and was avirulent on *B. oleracea* var. *capitata*. Race RM2 was isolated from *B. rapa* (= *B. campestris*) var. brown and yellow sarson and *Eruca sativa* and was avirulent on *B. oleracea* var. *capitata* and *B. oleracea* var. *botrytis*. Race V3 was virulent on all the eight host species tested. This race was isolated from vegetable host species like radish, cabbage and cauliflower (Table 9). This study indicated distinct pathotypes in *A. brassicae* infecting different *Brassica* species. According to Mridha (1983), thirteen isolates of *A. brassicae* tested on selected cultivars of winter oilseed rape differed in their virulence.

*Alternaria brassicicola* has been found to be more common on vegetable crops than on oil-yielding brassicas. Stoll (1952) characterized three isolates of this pathogen infecting siliquae of cauliflower seed crop as highly aggressive, less aggressive and non-pathogenic behaviour. However, highly aggressive isolates were less frequent (7.48%) than the moderately aggressive isolates (56.86%). Cultural and morphological variations in the isolates of this pathogen have been indicated with no distinction in pathogenic behaviour (Campbell, 1970a; Campbell *et al.*, 1968; Changsri and Weber, 1963). Spontaneous occurrence of Albino mutants of this fungus have been observed (Campbell, 1970a; Campbell *et al.*, 1968).

*Alternaria raphani* is the major pathogen of radish, but occurs on other brassicaceous hosts also. Atkinson (1953) obtained 312 isolates of this pathogen from different geographical areas and classified them as "Wild Type" and "Variant Type". He considered the former as being less virulent than the latter. No



differences were observed in their nutritional requirement for growth. In a later study Changsri and Weber (1963) did not find any variation in the *A. raphani* isolates obtained from different geographical areas of Canada on *B. nigra*, *B. napus* and *B. rapa*.

Table 9. Physiologic races of *Alternaria brassicae* (Saharan & Kadian, 1983)

| Host differential                      | <u>Races / pathotypes</u> |     |    |
|--|---------------------------|-----|----|
|  | RM1                       | RM2 | V3 |
| <i>Brassica juncea</i>                 | S*                        | S   | S  |
| <i>B. rapa</i> var. sarson             | S                         | S   | S  |
| <i>B. rapa</i> var. dichotoma          | S                         | S   | S  |
| <i>B. rapa</i> var. toria              | S                         | S   | S  |
| <i>Eruca sativa</i>                    | S                         | S   | S  |
| <i>Raphanus sativus</i>                | S                         | S   | S  |
| <i>Brassica oleracea</i> var. capitata | R*                        | R   | S  |
| <i>B. oleracea</i> var. botrytis       | S                         | R   | S  |

\*R = Resistant; S= Susceptible

#### d. Growth and Sporulation

i) **Culture Media:** A number of culture media have been reported suitable for growth and sporulation of *Alternaria* species pathogenic on brassicaceous plants (Table 10). According to Neergaard (1945), *A. brassicae* sporulates profusely on malt extract and standard nutrient agar, but very poorly on PDA. Atkinson (1950), on the other hand, found good sporulation on both PDA and malt agar. According to Ansari *et al.* (1988a), although *A. brassicae* grows and sporulates well on a wide range of media, PDA was found to be the best. Changsri (1960, 1961) found slightly poorer growth of *A. brassicae* in comparison to *A. brassicicola* and *A. raphani* which grew well on a wide range of agar media. On PDA, Mukadam and Deshpande (1977) found that *A. brassicae* not only grew and sporulated poorly, it also lost its ability to grow and sporulate with successive subculturing.

*Alternaria brassicae* from *Eruca sativa* grows best on glucose- asparagine agar medium and Richard's liquid medium. Mycelial growth is maximum in Richard's medium and least in Brown's medium. It is capable of utilizing nitrate, nitrite, ammonical and organic forms of nitrogen (Khandelwal *et al.*, 1970; Prasada *et al.*, 1970).

Single spore transfers of *A. brassicae* on 10% alfalfa decoction agar produces the largest amount of spores (McDonald, 1959). It grows well on pechay decoction



agar, corn meal agar, Czapek's agar, coconut agar, leonian agar, PDA, V-8 juice agar, oat meal agar, nutrient agar and prune agar, while sporulation is abundant on all but corn and oat meal agar (Lapis and Ricaforte, 1974). Billotte (1963) induced abundant sporulation of *A. brassicae* by the slow desiccation of culture in open petri dishes preceded by removal of the aerial mycelium and washing in running water. According to Degenhardt (1973), V-8 juice agar with Rose Bengal plus streptomycin stimulates sporulation of both *A. brassicae* and *A. raphani*.

According to Gupta *et al.* (1969), calcium nitrate and to a lesser extent potassium nitrate supports maximum growth of *A. brassicae* isolated from *B. oleracea* var. *botrytis*. Increased growth is correlated with the carbohydrate content of the medium. Buchwaldt *et al.* (1984) found that on Czapek's Dox medium, sinigrin (allylglucosinolate) does not influence growth rates of *A. brassicae*, but at increasing sinigrin concentrations, colonies become darker; on PDA, colonies are dark at all sinigrin concentrations. On PDA, sinigrin has no effect on growth rates in darkness, but in UV light, the high sinigrin concentration increases the mycelial growth slightly.

*Alternaria brassicicola* grows and sporulates well on a wide range of agar media (Changsri 1960, 1961), but shows marked selectivity in utilizing different carbon sources. Lactose is the best source of carbon for maximum growth followed by glycerol. The fungus prefers ammonical nitrogen over nitrate and nitrite. Growth of *A. brassicicola* is stimulated by DL-phenyl alanine, DL-valine, asparagine, glycine and DL-serine (Jain, 1974). The biosynthesis of lipids and phospholipids is best on Czapek's medium (Aizina *et al.*, 1976).

*Alternaria raphani* grows well in culture but sporulation is rather poor (Changsri, 1961). Spores of *A. raphani* are produced plentifully in most brassicaeous leaf decoction agar media, in which chlamydospores are produced abundantly. The density of the thallus and sporulation decreases as the amount of leaf decoction per litre is decreased from 400 to 6.5 g. Atkinson (1950) also reports abundant sporulation by wounding plate cultures and removing the lids of the culture plates. *A. raphani* does not require an exogenous vitamin source for growth, although early growth is stimulated by the addition of vitamins (Taber *et al.*, 1968). *A. raphani* grows faster when glutamic acid is supplied as a nitrogen source. Taber *et al.* (1968) in their extensive nutritional studies, report that *A. brassicae*, *A. brassicicola* and *A. raphani* grow well on most carbon sources; starch supports the most rapid growth, whereas methyl cellulose, filter paper strips and mannitol are very poor carbon sources.

Although the three species differ strikingly in their rates of utilization of mannitol, both *A. raphani* and *A. brassicicola* synthesize this polyol. Phenylalanine supports faster growth of *A. brassicicola* than other nitrogen sources, whereas glutamic acid and ammonium succinate stimulate more rapid growth of *A. raphani* and *A. brassicae* (Taber, 1964).

**ii) Temperature and Relative Humidity:** The optimum temperature for maximum sporulation of *A. brassicae* and *A. raphani* is between 23–25°C (Changsri and Weber, 1963; Degenhardt, 1973; McDonald, 1959; Neergaard, 1945; Singh,

1980; Taber, 1964). *A. brassicae* has more demanding growth requirements, with a

Table 10. Culture media for growth and sporulation of *Alternaria* species pathogenic on Brassicaceae (Verma and Saharan, 1993)

| Liquid / Solid Media          | Reference   |
|-------------------------------|---|
| Alfalfa decoction             | . . . McDonald, 1959  |
| Asthana and Hawker's          | . . . Gupta <i>et al.</i> , 1969  |
| Brown's starch                | . . . Ansari <i>et al.</i> , 1988; Gupta <i>et al.</i> , 1969; Prasada <i>et al.</i> , 1970 |
| Coconut                       | . . . Lapis and Ricaforte, 1974   |
| Corn meal                     | . . . Ansari <i>et al.</i> , 1988; Lapis and Ricaforte, 1974; Singh, 1980                   |
| Czapek's                      | . . . Gupta <i>et al.</i> , 1969; Lapis and Ricaforte, 1974; Prasada <i>et al.</i> , 1970   |
| <i>Eruca sativa</i> decoction | . . . Prasada <i>et al.</i> , 1970  |
| Glucose asparagine            | . . . Prasada <i>et al.</i> , 1970  |
| Houston                       | . . . Singh, 1980   |
| Kirchoff's                    | . . . Ansari <i>et al.</i> , 1988   |
| Leonian                       | . . . Lapis and Ricaforte, 1974   |
| Malt extract                  | . . . Ansari <i>et al.</i> , 1988   |
| Mustard leaf extract          | . . . Ansari <i>et al.</i> , 1988   |
| Oat meal                      | . . . Prasada <i>et al.</i> , 1970; Singh, 1980   |
| Pechay decoction              | . . . Lapis and Ricaforte, 1974   |
| Potato dextrose               | . . . Ansari <i>et al.</i> , 1988; Prasada <i>et al.</i> , 1970                             |
| Potato dextrose asparagine    | . . . Ansari <i>et al.</i> , 1988   |
| Potato sucrose                | . . . McDonald, 1959  |
| Rice meal                     | . . . Singh, 1980   |
| Richard's                     | . . . Ansari <i>et al.</i> , 1988; Gupta <i>et al.</i> , 1969; Prasada <i>et al.</i> , 1970 |
| Sabouraud's                   | . . . Ansari <i>et al.</i> , 1988; Prasada <i>et al.</i> , 1970                             |
| V-8                           | . . . McDonald, 1959  |
| Wheat meal                    | . . . Singh, 1980   |

distinct optimal growth peak at 22.5°C (Gupta *et al.*, 1972; Singh, 1980). The temperature growth optimum of *A. brassicicola* is 25–27°C (Sarkar and Sen Gupta, 1978), but growth continues to the extremes of 6°C and 37°C. According to Changsri (1960, 1961) and Changsri and Weber (1960, 1963), the optimum temperatures for growth in culture of *A. brassicicola*, *A. brassicae* and *A. raphani* are 24–28°C, 20–24°C, and 24–28°C, respectively. However, according to Taber *et al.* (1968), *A. raphani* and *A. brassicae* grow better between 20–25°C on malt agar, whereas *A. brassicicola* grows well over a wider temperature ranges. Lapis and Ricaforte (1974) report profuse mycelial growth and sporulation of *A. brassicae* at 16–24°C, but according to Ansari *et al.* (1989a), the optimum is 23°C.



*Alternaria brassicae* isolated from *C. abyssinica* grows well on malt agar and nutrient agar at 0.5–33°C with optimum growth at 23°C; sporulation is maximum at 20–30°C. *A. brassicicola* grows well between 0.5–38°C with an optimum growth at 25°C; sporulation is maximum at 17–30°C. *A. alternata* grows well from 0.5–40°C with optimum growth and sporulation at 25–27°C, and 20–30°C, respectively (Czyzewska, 1970).

According to Maude *et al.* (1986), at least 12 h of continuous high relative humidity and temperature of more than 14°C are required for abundant sporulation of *A. brassicae* and *A. brassicicola*. Below 14°C the sporulation is delayed under more humid conditions; at 8°C at least 30 h of high relative humidity is required for sporulation. Both fungi produce viable spores for 12 weeks on leaf and 20 weeks on stubble under field conditions. Ansari *et al.* (1989a), observed 95–100 percent relative humidity optimum for mycelial growth and sporulation of *A. brassicae*.

**iii) Hydrogen Ion Concentrations (pH):** The optimum pH requirement for growth and sporulation of *A. brassicicola*, *A. brassicae*, and *A. raphani* are 6.0–8.0, 7.1–8.0, and 7.1–8.0, respectively (Changsri, 1961; Changsri and Weber, 1963). According to Gupta *et al.* (1969), *A. brassicae* isolated from *B. oleracea* var. *botrytis* tolerates a wide pH range from 3.0–9.0, the optimum being 5.5. No sporulation occurs at pH levels below 3.0 and above 9.0. Very good sporulation occurs at pH levels between 5.0 and 6.5. According to Taber *et al.* (1968), *A. raphani* grows well over a pH range of 4.8 - 7.2. Mycelial growth occurs in pH range of 2.9–8.2 with an optimum at 6.5 (Ellis, 1968a,b; Ansari *et al.*, 1989a).

**iv) Light and Darkness:** Maximum growth and sporulation by *A. brassicae* occurs with alternating light and darkness. Continuous light completely inhibits sporulation. In light, a definite zonation of spore production occurs (Ansari *et al.*, 1989a; Changsri and Weber, 1963; Gupta *et al.*, 1972; Mridha, 1986; Mukadam and Deshpande, 1979a; Taber, 1964). According to Sasaki *et al.* (1985), sporulation of *A. brassicae* was inhibited by monochromatic radiation between 350 and 520 nm and reduced by continuous irradiation of UV radiation shorter than 350 nm.

Zone formation in cultures of *A. brassicicola* is affected by light. Three minutes of light exposure or six hours of darkness alternating from the original source of light is required to induce zonation. Spore development is plentiful under combinations of intermittent light and darkness (Changsri, 1961; Changsri and Weber, 1963). Taber (1964) found that the longer the wave length, the greater is the production of conidia in relation to chlamydospores by *A. raphani*.

#### e) Perpetuation

It is believed that *Alternaria* species infecting brassicaceous plants survive and perpetuate through infected seeds, diseased plant debris, pathogen propagules in the soil and cultivated/weed hosts in a particular agro-ecosystem (Chupp, 1925; Chupp and Sherf, 1960; Dixon, 1981; Ellis, 1968a,b, 1971; Humpherson-Jones, 1989; Kolte, 1985a,b; Neergaard, 1945; Putnam *et al.*, 1972; Saharan, 1992a; Sherf and McNab, 1986; Tsuneda and Skoropad, 1977b; Vaartnou and Tewari, 1972;



Verma and Saharan, 1993; Weber, 1973). Seed-borne *Alternaria* are capable of surviving as viable conidia and/or as internal mycelium for periods long enough for the seed to be harvested, stored, transported and finally sown. The proportions of *Brassica* seed carrying *Alternaria* spp. are very high. Richardson (1970) found 40 and 10%, respectively, of *Brassica* seed infected with *A. brassicicola* and *A. brassicae*. Infection levels up to 50% on *B. oleracea* var. *capitata* cultivar Houston Evergreen and up to 90% on turnip rape (*B. rapa*) are reported from Canada (Petrie, 1974). In the UK infected seed is the primary source of the *Brassica* dark leaf spot fungi *A. brassicae* and *A. brassicicola* in autumn sown oilseed rape and vegetable brassicas (Humpherson-Jones, 1985; Gladders, 1984). In oilseed rape, *A. brassicae* is the dominant species, but in vegetable *Brassica* seeds *A. brassicicola* predominates. Although seed of vegetable brassicas including brussels sprouts may be infected with *A. brassicicola*, *A. brassicae* has not been detected in the seed of this crop (Humpherson-Jones, 1985). Nevertheless, *A. brassicae* has recently assumed increasing dominance as a pathogen of brussels sprouts. However, in tropical countries like India, *A. brassicae* gets eliminated from the seeds of oilseed brassicas during storage from April to September at 25–35°C (Table 11) temperature (Chahal, 1981; Kolte, 1985a; Saharan, 1992a). *A. raphani* is internally seed-borne in all parts of dormant radish seed (Atkinson, 1950). According to Dixon (1981), infected seed is the main avenue of transmission of three important *Alternaria* spp. of brassicas. This can take place both externally as conidia adhering to the testa, or as internal mycelia present within the seed tissues. The latter may lead to complete destruction of the embryo. Four phases of seed transmission can be distinguished: 1) transmission from seed to developing seedlings, 2) transmission from seed to adjacent seed, 3) transmission from adult plant to seed by fungal growth through the green siliquae coat into the moist atmosphere with the seed pod, and 4) transmission from adult plant to adult plant.

Table 11. Survival of *Alternaria brassicae* in rapeseed-mustard seed during storage at Ludhiana and Hisar in 1979 (Saharan , 1984)

| Months    | PERCENT SEED INFECTION |         |               |
|-----------|------------------------|---------|---------------|
|           | RLM-198                | Prakash | Yellow sarson |
| April     | 30.0                   | 11.8    | 15.5          |
| May       | 24.0                   | 1.7     | 1.9           |
| June      | 16.0                   | 0.0     | 0.0           |
| July      | 10.0                   | 0.3     | 0.0           |
| August    | 0.0                    | 0.0     | 0.0           |
| September | 0.0                    | 0.0     | 0.0           |

In the Philippines, *A. brassicae* remains viable for 12–14 months in infected leaves (Lapis and Ricaforte, 1974). According to Tripathi and Kaushik (1984), *A. brassicae* overwinters with plant debris buried in the field below the depth of 7.5

cm. Ansari *et al.* (1989b), observed that in temperate and subtropical countries, *A. brassicae* survives and perpetuates through diseased plant debris. In the UK, infected debris of *B. oleracea* seed crop is shown to be a major source of *A. brassicicola* spores (Humpherson-Jones and Maude, 1982a). Infected debris of *Brassica* crops, with *A. brassicae* and *A. brassicicola* remaining on the ground after harvest, may provide a source of dark leaf spot infection which may be implicated in the spread of the disease within and between crops (Humpherson-Jones, 1989). On leaves of oilseed rape and cabbage the fungi produce viable spores for as long as the leaf tissue survive which is up to 6 weeks in winter oilseed rape, and up to 8 weeks in summer. On cabbage spore viability lasts up to 8 weeks in winter and up to 12 weeks in summer (Humpherson-Jones and Hocart, 1983).

Infected Crambe seed is the main source of primary inoculum of *A. cincinans* (Holcomb and Newman, 1970). The virulence of *A. brassicicola*, also from Crambe, can be retained for one year by culturing on filter paper and storage in petri dishes at 5°C (Kilpatrick, 1975, 1976).

*Alternaria raphani* is reported to survive for five years in soil culture (Atkinson, 1950, 1953). The fungus shows no change in cultural characteristics after its five year period in dry soil, nor any loss of virulence when plants of radish, stock and wallflower are inoculated (Atkinson, 1953). It is also possible that *A. brassicae* and *A. raphani* survive through chlamydospores (Atkinson, 1953; Tsuneda and Skoropad, 1977b; Vaartnou and Tewari, 1972). According to Vaartnou and Tewari (1972), hyphal chlamydospores of *A. raphani* are produced on the stem, siliquae, and seed of infected plants. Such spores have been found to remain viable even after prolonged deep freezing of the infected material. Tsuneda and Skoropad (1977b) observed that conidia of *A. brassicae* are transformed into microsclerotia (Fig. 9, 10). Such structures are round, darkly pigmented, resist dessication, and function in a similar manner to those of the sclerotia produced by other fungi. More microsclerotia are formed only on the previously affected and partially decayed plant tissues suggesting the possibility of survival of the fungus through such structures. These structures on germination have been found to produce numerous new conidiophores and conidia.

Numerous brassicaceous weeds, forage brassicas and other weed hosts also serve as sources of primary inoculum of *Alternaria* spp. infecting several economically important brassicas (Table 12).

#### **f) Spore Germination**

**i) Effect of Culture Media:** The germinability of spores of *A. brassicae* and *A. brassicicola* is higher when cultivated on rich media than the spores produced on complex or poorly nutritive media. The presence of metabolic inhibitors in the growth media reduces spore germinability even in the presence of nutrients (Czapek's-Dox agar) and causes abnormalities in their morphology. Spores of both the species show maximum germinability irrespective of their age (up to 20 days). Increase in the age of the spores (30 days onwards) either increases their latent period and/or reduces their germinability, even in the presence of nutrients (Gupta *et al.*, 1969).

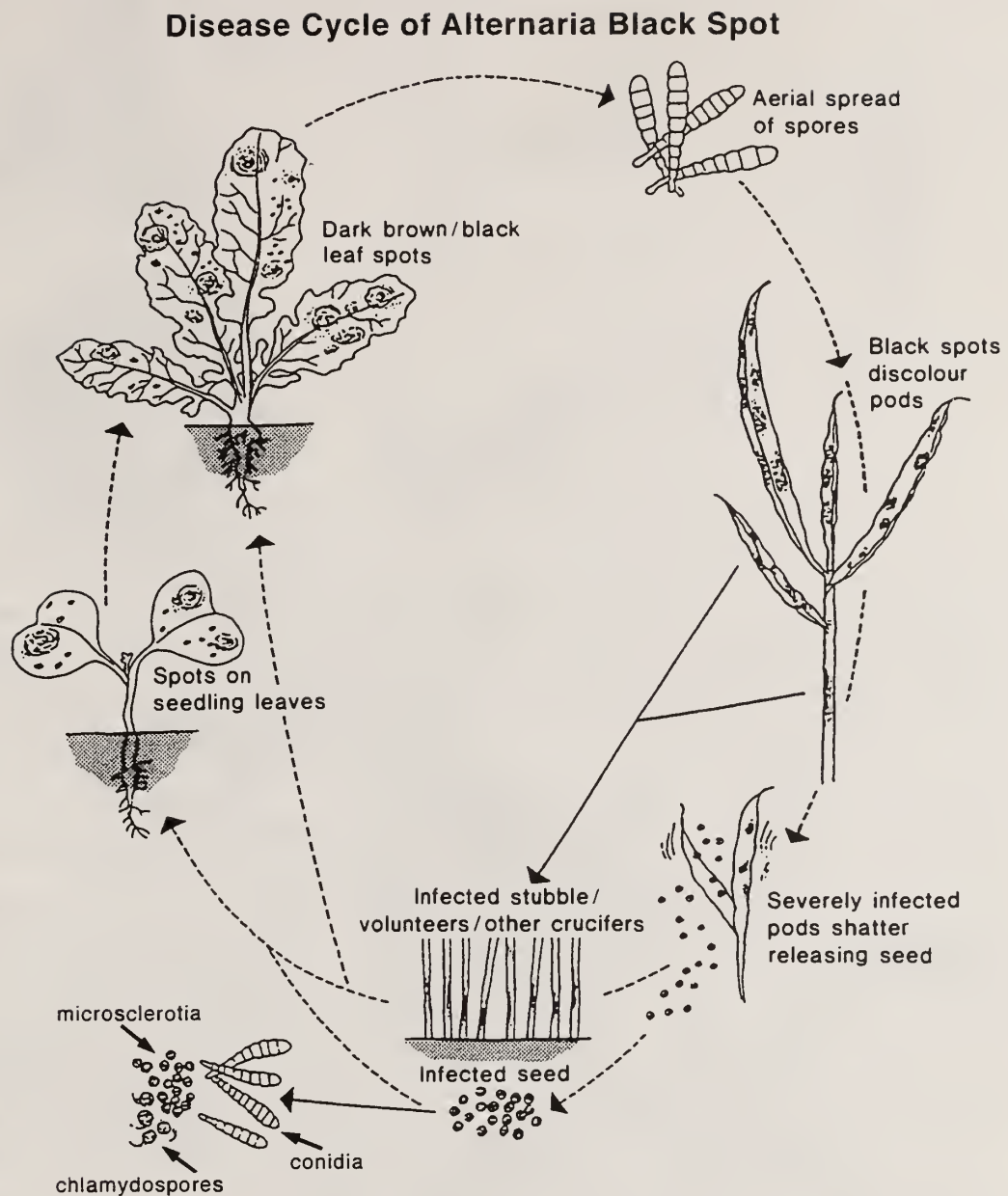


Fig. 9. Life cycle of *Alternaria* diseases on crucifers (Courtesy: Thomas, 1984).





Fig. 10. Light micrographs showing the development of a microsclerotium from *Alternaria brassicae* conidium: (a) conidium; (b) initial stage in the formation of a microsclerotium; (c) half developed microsclerotium with about 50 cells; (d) mature microsclerotium still showing head and beak of the original conidium. x 400; (e) germination of a mature microsclerotium to form hyphae. x 400; and (f) germination of a frozen-thawed microsclerotium showing many new conidia. x 650 (Courtesy: Tsuneda and Skoropad, 1977).

**ii) Effect of Temperature and Relative Humidity:** In *Alternaria brassicae* causing brown rot of cauliflower the optimum temperature for spore germination is 32–35°C with the minimum of <1.5°C and the maximum of 40–46°C; as no temperature between 40.5°C and 46°C was tried, the exact maximum temperature was not determined. The optimum temperature for mycelial growth is 25–27°C (Weimer, 1924). Degenhardt *et al.* (1982) observed excellent germination of *A. brassicae* spores at 15°C with 90% relative humidity and 6–8 hours of host leaf wetness period in contrast to very poor germination of *A. brassicicola* spores under similar conditions. Gupta *et al.* (1972) found 17–19°C to be optimal for conidial germination of *A. brassicae*. According to Ansari *et al.* (1988b), *A. brassicae* spores germinate over a very wide range of 10–28°C with an optimum of 23°C. However, Kadian and Saharan (1984) recorded 25°C as the optimum temperature for spore germination of *A. brassicae*. Spore germination commences after 4 hours and reaches its peak after about 24 hours; relative humidity of >90% is essential for maximum spore germination.

Conidial germination of *A. brassicicola* is optimal at 30°C (Gupta *et al.*, 1972). Sarkar and Sengupta (1978) observed that conidia of *A. brassicicola* germinate best at 90–100% relative humidity and a temperature of 22–32°C. Self inhibition of *A. brassicicola* occurs when the spore load in the germination medium is 0.2 optical density. The self-inhibiting compound is volatile at 35°C and denatures at 90°C, and is thought to be a substance of low molecular weight (Mukadam, 1982). Barton and Fine (1958) obtained 86–90% spore germination of *A. brassicicola* in the presence of 200 ppm gibberellic acid.

Conidia of *A. alternata* causing leaf and pod blight of radish germinate at 15–30°C with an optimum at 20–25°C (Singh and Suhag, 1983).

**iii) Effect of Host Extract and Exudates:** Leaf extracts of *Brassica* spp. are generally inhibitory to spore germination of *A. brassicae*. Leaf extracts and exudates of both susceptible and resistant cultivars are inhibitory but inhibitory effects of resistant cultivars like Tower and RC781 are more pronounced; generally, leaf exudates are more inhibitory than leaf extracts (Kadian and Saharan, 1984). The effect of leaf exudates of yellow sarson (*B. rapa*) and taramira (*E. sativa*) is reported to vary with the host variety, age of host plant and maturity of the leaves (Sharma *et al.*, 1985b).

**iv) Effect of Light Intensity:** Direct exposure to strong light intensity is deleterious to *A. brassicae* spores, inhibiting germination and reducing host infection (Kadian and Saharan, 1984). Maximum sporulation of *A. brassicae* occurs with alternating light and darkness. Continuous light completely inhibits sporulation (Ansari *et al.*, 1989a; Changsri and Weber, 1963; Gupta *et al.*, 1972; Mridha, 1986; Mukadam and Deshpande, 1979a; Saski *et al.*, 1985; Taber, 1964).

#### **g. Seed Infection**

*Alternaria* blight has been reported to be a serious seed-borne disease of several seed producing brassicaceous crops in many countries of the world (Crosier and Patrick, 1940; Ellis, 1968a,b; Green, 1947; Groves and Skolko, 1944; Holcomb



and Newman, 1970; Holtzhausen, 1978; Holtzhausen and Knox-Davies, 1974; Jouan *et al.*, 1972; Kilpatrick, 1975, 1976; Kolte, 1985a,b; Kothanur *et al.*, 1982; Nipoti, 1978; Noble and Richardson, 1968; Petrie, 1974; Pound *et al.*, 1951; Richardson, 1970, 1979; Schimmer, 1953; Van Schreven, 1953; Verma and Saharan, 1993; Wiltshire, 1947). High recovery of *A. raphani* from radish seeds in Michigan (McLean, 1947), and *A. brassicicola* from cabbage seeds in Washington (Pound *et al.*, 1951), has been reported. In South Africa, all three species of *Alternaria* (*A. brassicae*, *A. brassicicola* and *A. raphani*), have been isolated from seeds of cabbage, cauliflower, broccoli and brussels sprouts (Holtzhausen and Knox-Davis, 1974; Knox-Davies, 1980). In Egypt, testing for seed health in cabbage, cauliflower, turnip and radish showed the presence of *A. brassicicola* and *A. raphani* (Michail *et al.*, 1979). In Finland 91% of white cabbage and red cabbage seed lots were found to be infected with *A. brassicicola*. *A. brassicae* was found in 4% of the cabbage and in 31% of the rape seed lots. *A. raphani* was found in 30% of the radish and black radish seed lots (Tahvonen, 1979).

In the UK between 1981–1984, up to 25% of *B. napus* seeds, and 8.5% of *B. rapa* (turnip) seeds yielded *A. brassicae*, and 55% and 13% seeds of *B. oleracea*, respectively, yielded *A. brassicicola* and *A. brassicae* (Humpherson-Jones, 1985). *A. brassicae* was detected at varying levels on seed of rape harvested from different climatic districts of New South Wales during 1983. The highest levels were on seed samples from the South West slopes (North) and Riverina (av. 22.8 and 14.2%, respectively) with much lower levels of infection (av. <8.0%) on samples from central and Northern districts (Stovold *et al.*, 1987). In Scotland, up to 89% of seeds of *B. napus* were recorded as being infected with *A. brassicae* (Kothanur *et al.*, 1982).

In Canada, Petrie (1974) reported *A. brassicae* and *A. raphani* as important seed-borne pathogens of oleiferous brassicas. *B. rapa* (turnip rape) had higher levels of *A. brassicae* than *A. raphani*; *B. napus* (rape) seed contained considerably lower levels of both species. In heavily infested seed of *B. rapa* 73% of the *A. brassicae* and 90% of the *A. raphani* occurred on the seed surface. Storage of infected seed for 6–8 months at 25°C reduced the levels of infestation by more than 50%. In rapeseed-mustard the frequency of infection of seed by *A. brassicae* varies with the number of lesions on the siliquae. Seed infection diminished with the length of storage period at higher temperature in summer months (Chahal, 1981). Mustard seeds showing 42% infection of *A. brassicae* in April became completely free when stored at 45 and 35°C for two and three months, respectively (Shivpuri and Siradhana, 1989). According to Vishnuavat *et al.* (1985), *A. brassicae* seed infection in mustard was eliminated during storage from March to July at 11.4–39.2°C, but survived at 5°C in cold storage.

*Alternaria* species comprised 86% of the fungal flora isolated from samples of Crambe seed from Illinois, Indiana, Maryland and Ohio; *A. tenuis* was isolated from 47% and *A. brassicicola* from 39% of the samples (Kilpatrick, 1976). The high percentage of *A. brassicicola* infected seed indicates that infection is internal, and that the fungus is capable of systemic infection and direct penetration of pods.

The recovery of *Alternaria* spp. is better through the blotter method than



the agar plate method. Ashraf and Chaudhary (1989) recovered 20.8% of *A. alternata* from rapeseed seed on blotter and 10.7% on Agar plate. The recovery of *A. brassicae* was 10.7% and 10.5%, respectively. Jain *et al.* (1982) recovered 31.5–10.5% *A. alternata* and 6.0–1.5% *A. brassicae* from rapeseed seed through the blotter method but none through the Agar plate method.

Domsch (1957) described the infection of rape and cabbage seeds by *A. brassicae* and *A. brassicicola*. The relationship between *A. brassicicola* and *Brassica* seeds was determined by Knox-Davies (1980). The fungus first colonizes the testas of inoculated seeds as they rupture, and then attacks embryos after a further 24–30 hours. Testas of naturally-infected cabbage seeds are colonized prior to rupture and largely in the hilum area. Spores lodge in the cavities and folds of tissue depressions at the hilum and micropyle. Spore germination and hyphal development are frequently poor at the hilum, but in some cases mycelial development is extensive over the hilum. Development on the remainder of the testa is generally poor, the only extensive growth being present on damaged seeds (Fig. 11). According to Vannacci and Pecchia (1988), *A. raphani* occurs in all seed parts of the radish and conidia are present on the seed coat. Similar observations were made by Atkinson (1950).

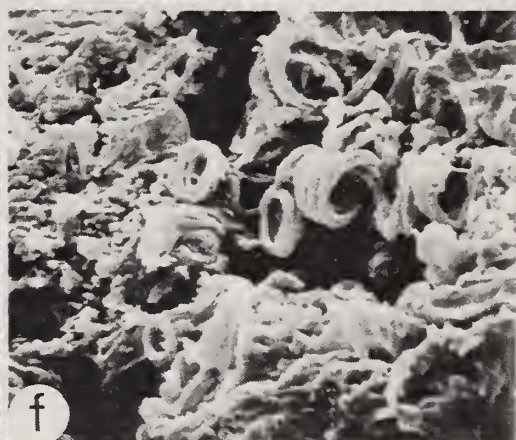
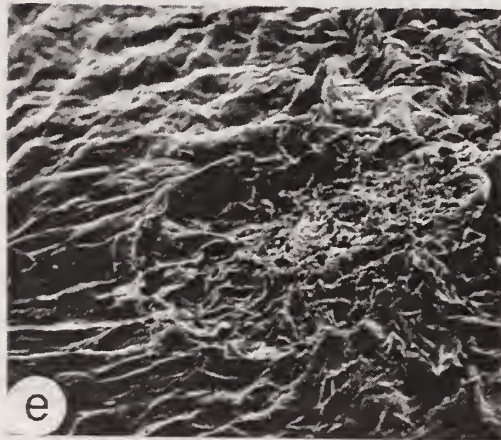
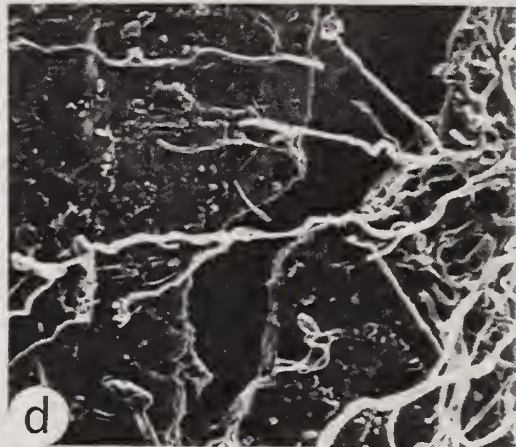
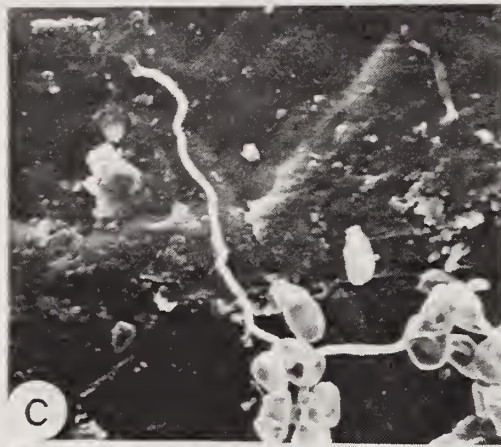
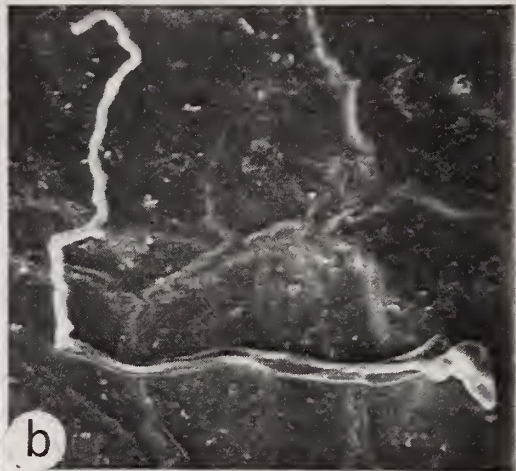
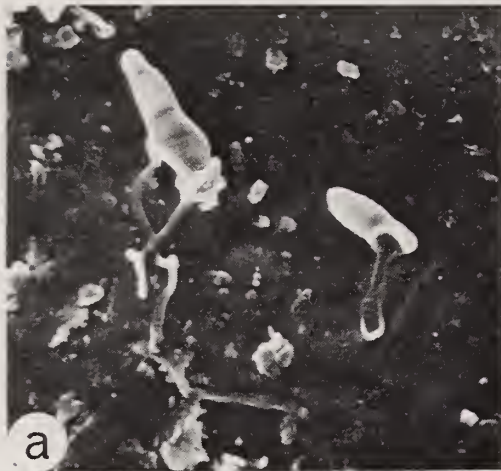
#### 4. HOST RANGE

*Alternaria* species are world wide and attack a large number of brassicaceous crops. Among the common hosts of economic importance are rapeseed- mustard, cabbage, chinese cabbage, cauliflower, broccoli, horse radish, turnip, radish, brussels sprouts, collards, kohlrabi, rutabaga, and swedes. Apart from these, the inventory of hosts reported to be infected by *A. brassicae*, *A. brassicicola*, *A. raphani* and *A. alternata* is given in Table 12 (Ansari *et al.*, 1990a; Atkinson, 1950; Chupp, 1925; Czyzewska, 1969; Ellis, 1968a,b, 1971; Kadian and Saharan, 1983; Kolte, 1985a,b; McDonald, 1959; Putnam *et al.*, 1972; Rai and Sinha, 1963; Saharan *et al.*, 1982; Tewari and Conn, 1993; Verma and Saharan, 1993; Walker, 1927; Weber, 1932).

#### 5. DISEASE CYCLE

The pathogen survives or overwinters on infected crop residues, seed and brassicaceous weeds. Infections on host plants begin directly from infested seed, from spores produced on crop residues, from infections on brassicaceous weeds or possibly from microsclerotia and chlamydospores if produced on infected debris (Figs. 9, 10). Spores are produced abundantly in wet weather and are dispersed locally by rain splash and wind. Spore release, which is passive, is stimulated by

Fig. 11. Scanning electron micrographs of the surfaces of cabbage seeds inoculated with *Alternaria brassicicola* and germinated on water agar for 24 h at 21°C: (a) germinated spores with collapsed germ-tubes and initials of turgid aerial hyphae. x 1,200; (b) germinated spore with collapsed germ-tube and turgid aerial hyphae. x 1,000; (c) turgid aerial germ-tube with collapsed tip. x 1,000; (d) profuse development of hyphae on damaged seed. x 500; (e) roughened hilum area with broken vascular elements of the funiculus. x 200; and (f) spiral-thickening of xylem vessels of the broken vascular elements of the hilum shown in Fig. e. x 2,000 (Courtesy: Knox-Davis, 1979).





falling humidity and restricted by a high humidity so that air spore concentration in a diseased crop exhibits a distinct diurnal periodicity, with maximum concentration occurring in the early afternoon and minimum concentration in the early morning (Kolte, 1985a,b; Saharan, 1992a; Smith *et al.*, 1988; Thomas, 1984; Walker, 1952). In the areas where crops are grown sequentially, *A. brassicae* overwintering saprophytically on diseased plant debris is the major source of primary inoculum for new crops. In Sweden, in areas where only summer crops of rape are grown, the disease is less severe than in areas where summer and winter

Table 12. Host species susceptible to *Alternaria brassicae*, *Alternaria brassicicola*, *Alternaria raphani* and *Alternaria alternata* (Verma and Saharan, 1993)

| Host  | Reference                    |
|---|------------------------------|
| <b>A. brassicae</b>                             |                              |
| <i>Armoracia rusticana</i>                      | Horse radish                 |
| <i>A. lapathifolia</i>                          | Tewari and Conn, 1993        |
| <i>Anagallis arvensis</i>                       | Bolle, 1924                  |
| <i>Brassica oleracea</i> var. <i>gongylodes</i> | Saharan <i>et al.</i> , 1982 |
| Kohlrabi  | Weiss, 1960                  |
| <i>B. oleracea</i> var. <i>viridis</i>          | Kale                         |
| <i>B. napo brassica</i>                         | Weiss, 1960                  |
| <i>B. oleracea</i> var. <i>gemmifera</i>        | Rutabaga                     |
| <i>B. oleracea</i> var. <i>botrytis</i>         | Brussels sprouts             |
| <i>B. oleracea</i> var. <i>capitata</i>         | Weiss, 1960                  |
| <i>B. napus</i> ssp. <i>oleifera</i>            | Cauliflower                  |
| <i>B. tournifortii</i>                          | Weiss, 1960                  |
| <i>B. nigra</i>                                 | Cabbage                      |
| <i>B. chinensis</i>                             | McDonald, 1959               |
| <i>B. pekinensis</i>                            | Rapeseed                     |
| <i>B. rugosa</i>                                | McDonald, 1959               |
| <i>B. rapa</i> var. brown sarson                | Wild mustard                 |
| <i>B. rapa</i> var. yellow sarson               | Kadian and Saharan, 1983     |
| <i>B. rapa</i> var. toria                       | Kadian and Saharan, 1983     |
| <i>B. hirta</i>                                 | Black mustard                |
| <i>B. kabar</i>                                 | Chinese cabbage              |
| <i>B. rapa</i> ssp. <i>oleifera</i>             | - do -                       |
| <i>B. rapa</i> ssp. <i>rapifera</i>             | - do -                       |
| <i>B. carinata</i>                              | - do -                       |
|   | - do -                       |
|   | - do -                       |
|   | - do -                       |
|   | - do -                       |
|   | - do -                       |
|   | - do -                       |
|   | White Mustard                |
|   | - do -                       |
|   | White Mustard                |
|   | Weiss, 1960                  |
|   | Canola                       |
|   | Tewari and Conn, 1993        |
|   | Turnip                       |
|   | Tewari and Conn, 1993        |
|   | Abyssinian                   |
|   | - do -                       |
|   | mustard                      |
| <i>B. orientalis</i>                            | Bolle, 1924                  |
| <i>B. japonica</i>                              | Banga <i>et al.</i> , 1984   |
| <i>B. rapa</i>                                  | Rai and Sinha, 1963          |
| <i>B. juncea</i>                                | Mehta <i>et al.</i> , 1950   |
| <i>B. albo-glabra</i>                           | Seow and Lim, 1969           |



*Chenopodium album*  
*Cleome ciliata*  
*Convolvulus arvensis*  
*Citrus aurantium*  
*Cyamopsis psoraloides*  
*Crambe abyssinica*  
*C. maritima*  
*Cheiranthus cheirii*  
*Eruca sativa*  
*Erysimum cheiranthoides*

*Iberis amara*  
*I. umbellata*  
*Isatis tinctoria*  
*Lallemantia iberis*  
*Lepidium sativum*  
*L. latifolium*  
*Lactuca sativa*  
*Lunaria annua*  
*Matthiola incana*  
*Portulaca oleracea*  
*Rapahanus sativus*  
*Sinapis alba*  
*S. arvensis*  
*Sisymbrium officinale*  
*S. altissimum*  
*S. orientale*  
*Thlaspi arvense*

#### **A. brassicicola**

*B. oleracea* var. *capitata*  
*B. oleracea* var. *botrytis*  
*B. oleracea* var. *caularapa*  
*B. juncea*  
*B. pekinensis*  
*B. rapa*  
*B. carinata*  
  
*C. abyssinica*  
*E. sativa*  
*Godetia hybrida*  
*I. umbellata*  
*Lunaria rediviva*  
*R. sativus*

#### **A. raphani**

*B. rapa* var. *toria*  
*B. juncea*  
*B. oleracea* var. *botrytis*

Guar  
Crambe  
Crambe  
Wallflower  
Taramira  
Wormseed  
mustard  
Candytuft

Garden cress

Lettuce  
Honesty  
Stock

Radish  
White mustard  
Wild mustard  
Hedge mustard  
Tumbling weed

Stinkweed

Tripathi and Kaushik, 1984  
Lapis and Ricaforte, 1974  
Saharan *et al.*, 1982  
Rao, 1977  
Siddiqui, 1963  
Czyzewska, 1970  
Leppik, 1973  
McDonald, 1959  
Kadian and Saharan, 1983  
Tewari and Conn, 1993

McDonald, 1959  
Neergaard, 1939  
Bolle, 1924  
Darpoux, 1945  
McDonald, 1959  
McDonald, 1959  
Lapis and Ricaforte, 1974  
Baker and Davis, 1950  
Ware, 1936  
Lapis and Ricaforte, 1974  
McDonald, 1959  
Tewari and Conn, 1993  
- do -  
Putnam *et al.*, 1972  
- do -  
Anonymous, 1959  
Petrie and Vanterpool, 1966

Cabbage  
Cauliflower

Mustard  
Chinese cabbage  
Turnip  
Abyssinian  
mustard

Crambe  
Taramira

Radish

Rai and Sinha, 1963  
- do -  
- do -  
Bolkan *et al.*, 1983  
- do -  
Jamil, 1966  
Galvez and Romero, 1988  
  
Czyzewska, 1969  
Sharma, 1985  
Anonymous, 1946  
Neergaard, 1948  
Negru, 1959  
Rao, 1977

Rai and Sinha, 1963  
- do -  
Atkinson, 1950

Indian Mustard  
Cauliflower

|   |            |                             |
|---|------------|-----------------------------|
| <i>B. oleracea</i> var. <i>capitata</i> | Cabbage    | - do -                      |
| <i>B. napabrossica</i>                  | Swedes     | - do -                      |
| <i>Cheiranthus cheirii</i>              | Wallflower | - do -                      |
| <i>E. sativa</i>                        | Taramira   | Rai and Sinha, 1963         |
| <i>I. amara</i>                         | Candy tuft | Atkinson, 1950              |
| <i>I. armena</i>                        |            | Narian <i>et al.</i> , 1982 |
| <i>Lactuca sativa</i>                   | Lettuce    | Atkinson, 1950              |
| <i>M. incana</i>                        | Stock      | Baker and Davis, 1950       |
| <i>T. arvense</i>                       | Stinkweed  | Petrie and Vanterpool, 1966 |

#### ***A. alternata***

|  |                |                       |
|--|----------------|-----------------------|
| <i>B. rapa</i> var. brown sarson         |                | Singh and Suhag, 1983 |
| <i>B. rapa</i> var. toria                |                | - do -                |
| <i>B. juncea</i>                         | Indian Mustard | - do -                |
| <i>B. oleracea</i> var. <i>capitata</i>  | Cabbage        | - do -                |
| <i>B. oleracea</i> var. <i>botrytis</i>  | Cauliflower    | Rai and Sinha, 1963   |
| <i>B. oleracea</i> var. <i>caulorapa</i> |                | - do -                |
| <i>Beta vulgaris</i>                     | Spinach        | Singh and Suhag, 1983 |
| <i>Convolvulus arvensis</i>              | Hirankhuri     | - do -                |
| <i>C. abyssinica</i>                     | Crambe         | Czyzewska, 1969       |
| <i>E. sativa</i>                         | Taramira       | Rai and Sinha, 1963   |
| <i>I. amara</i>                          |                | - do -                |
| <i>Lepidium sativum</i>                  |                | - do -                |
| <i>Lycopersicon esculentum</i>           | Tomato         | Singh and Suhag, 1983 |
| <i>R. sativus</i>                        | Radish         | Rai and Sinha, 1963   |
| <i>Trianthema monogyna</i>               | Santhi         | Singh and Suhag, 1983 |

crops form a continuous green bridge. In the U.K. *A. brassicae* spores released from infected rape crops during harvest are widely dispersed and are potential agents of infection for other crops (Humpherson-Jones, 1984).

Although all four species of *Alternaria* i.e. *A. brassicae*, *A. brassicicola*, *A. raphani* and *A. alternata* occurring on Brassicaceae hosts are reported to be seedborne (Chahal and Kang, 1979a,b; Czyzewska, 1970; Knox Davies, 1980; Maude and Humpherson-Jones, 1980a,b; Rangel, 1945), it hardly plays any role in survival and dissemination of this pathogen in tropical countries such as India where summer temperatures often reach above 35°C. In the plains of India, seeds having more than 80% infection in April become completely free from infection in September after storage at 35°C (Chahal, 1981; Kolte, 1985a,b; Shivpuri and Siradhana, 1989; Tripathi and Kaushik, 1984). However, infected seed is the most important source of primary inoculum (19% internal seed infection in England) in cooler regions of the world (Smith *et al.*, 1988).

*Alternaria* spores from their courts of survival germinate in free water, penetrate host tissues through stomata and cause infection. The optimum

temperature for spore germination, mycelial growth and infection of rape by *A. brassicae* are 17–24°C, when severe infection may occur within 6 h. However, at the lower temperature of 12°C, infection is severely restricted, and only low levels of the disease occur within 15 h (Louvet and Billotte, 1964). *A. brassicicola* has a higher temperature requirement for spore germination (33–35°C), mycelial growth (25–27°C) and infection (28–31°C) than *A. brassicae* which may explain its more restricted occurrence. Free water on plant surface is required for infection with minimum wetting period of 6–16 h for initiation of the disease, and 48–72 h wetting period for maximum disease development (Humpherson-Jones, 1983; Kadian and Saharan, 1984; Rangel, 1945). Under favourable environmental conditions, lesions develop and produce wind-borne spores which may cause more infection on the same or neighbouring plants. The cycle continues throughout the season when conditions are favourable. Seeds may become infected following development of lesions on the pods. At harvest time, spores produced on stems, branches and pods may also infest the seed in the combine. Many brassicaceous hosts, when present as weeds in cultivated crops, help the pathogen to survive from year to year (Fig. 9).

## 6. PROCESS OF INFECTION AND PATHOGENESIS

The primary infection results from the wind borne spores produced on debris of previous crop or on weeds and other collateral hosts growing in the near vicinity. Seed as a source of primary inoculum is important in temperate regions. Chlamydospores and microsclerotia may initiate primary infection of *A. raphani* and *A. brassicae* (Figs. 9, 10). Conidia readily germinate in the presence of moisture by giving rise to a germ tube which emerges from any cell of the spore. Theoretically only one spore can cause infection in the brassicaceous seedlings indicating a threshold infection value at a very low level (Czyzewska, 1971). Germ tubes from germinated spores of *A. brassicae*, *A. brassicicola*, *A. raphani* and *A. alternata* generally penetrate the undamaged tissues of many brassicaceous hosts directly (Changsri and Weber, 1963; Czyzewska, 1971; Minkevicius, 1932; Neergaard, 1945; Van Schreven, 1953; Weimer, 1926), although indirect penetration through stomata has been reported in *A. brassicae* (Changsri, 1961; Tsunedo and Skoropad, 1978a).

*Alternaria* conidia are thought of as "dry conidia" which are wind dispersed, maximum release taking place in the mid-afternoon. There is no report on the numerical threshold for infection, consequently lesions may be initiated from a single germinating conidium. Maude and Humpherson-Jones (1977) found that infected marrow stem kale (*B. oleracea* var. *acephala*) liberates 50 spores per cubic meter of air just prior to harvesting, 190 spores per cubic meter of air at cutting, and 3200 spores per cubic meter of air when windrowed crops are harvested. Spores can be carried up to 1000 m downwind during harvesting. Sharma and Gupta (1978c) trapped *A. brassicae* and *A. brassicicola* spores from the air during October to April on brown sarson crop fields under Indian conditions. Conidia of *A. brassicae* were intercepted at all hours of exposure but conidia of *A. brassicicola* were seen only for a specific period during the day. Maximum conidial catch was observed in March which coincides with the maximum manifestation of disease in the field.

Frequent infection occurs when water is present on the host surface due to



dew formation. The infection processes of both *A. brassicae* and *A. brassicicola* are initiated if water is present for 5–8 h. For infection of *A. brassicae* in cauliflower leaves and heads, 25–31°C temperature is reported as optimum (Weimer, 1924); the disease increases with the increase in humidity of the surrounding air. In rapeseed, *A. brassicae* establishes itself in less than 6 h at 22°C (Louvet and Billotte, 1964). For infection of cabbage plants, both *A. brassicae* and *A. brassicicola* require free water with an optimum temperature of 25°C for *A. brassicicola*, and 15°C for *A. brassicae*. At these temperatures a minimum of 16 h is necessary for the initiation of infection, and 48–72 h for optimal disease development. Alternating wet and dry (70–80% RH) periods of 16 h and 8 h, respectively, restrict infection by both species. The differential temperature requirements of the two species become most marked at 10°C and 96 h of incubation after inoculation; *A. brassicicola* fails to produce significant infection, whereas infection by *A. brassicae* produces numerous lesions (Humpherson-Jones and Hocart, 1983).

With *A. brassicicola* cuticular penetration is most frequent and stomatal penetration occurs to a lesser extent. Penetration is preceded by formation of appressoria from the tip of germ tubes and are either spherical or club-shaped and 3–8 µm in diameter. Stomatal penetration is more common with *A. brassicae*. Once within the host, the epidermal cells are fully invaded and mycelia ramify through and between the mesophyll and palisade cells, the entire leaf is soon parasitized. Early in the post-penetration phase, the invaded epidermal cells become necrotic, and the parenchyma tissues ahead of the advancing hyphae often collapse (Dixon, 1981).

On the leaves of *B. napus* cultivar Midas and *B. rapa* cultivar Torch, conidia of *A. brassicae* germinate at the rate of 12.1% and 19.5%, respectively, at 9 h after inoculation. Conidia usually germinate by producing either germ tubes or secondary conidia. Penetration of leaves by *A. brassicae* is abundant at 24 h and occurs either with or without the formation of appressoria. Penetration of the cultivar Torch leaves occurs either directly through epidermal cells or indirectly through stomata, while Midas leaves are penetrated almost exclusively through the stomata. Black spot lesions develop within 48 h after inoculation (Tsunedo and Skoropad, 1978b). According to Tewari (1983, 1986), *A. brassicae* in rapeseed becomes subcuticular after direct penetration. This is followed by colonization of the epidermal and the mesophyll cells. In the leaves of rapeseed cultivars Candle and Altex the pathogen heavily colonizes the necrotic centre and is not present in the chlorotic area indicating that a diffusible metabolite may be directly or indirectly responsible for leaf chlorosis. The plasma membrane is the first target of the diffusible metabolite. Subsequently, the chloroplasts are either directly or indirectly affected leading to leaf chlorosis. Relative to chloroplasts, the effect on mitochondria is seen at a much later stage. The cells in the necrotic area are almost completely devoid of cellular organelles and reveal electron dense lamellar deposits (Figs. 12, 13; Tewari, 1983). *Alternaria* infection in the *C. abyssinica* leaves is preceded by toxic substances secreted by the fungus (Czyzeweska, 1971). The same may be true for other brassicas. According to Suri and Mandahar (1984, 1985, 1986) and Suri *et al.* (1983), cytokinin-like substances appear to be actively involved in infection and pathogenesis of *A. brassicicola*. Poapst *et al.* (1979) suggested the role of the hormone ethylene in screening cabbage leaves infected with *A.*

*brassicicola*. Production of cellulases and pectolytic enzymes by *A. brassicae* causing leaf blight disease of rapeseed-mustard has been observed (Nehemiah and Deshpande, 1976; Suri and Mandahar, 1982). The differential susceptibility of the brassicaceous hosts to *A. brassicae*, *A. brassicicola* and *A. raphani* has been correlated with differences in amino acid content (Changsri, 1961). The population of the pathogens varies with the age and variety of the host, maturity of leaves and climatic conditions. The colonies increase when the temperature and relative humidity of the field is 21.5°C and 85%, respectively. Both *A. brassicae* and *A. brassicicola* occur in greater profusion on leaves of *B. rapa* var. yellow sarson than on leaves of *E. sativa*. The young leaves have lower incidence of disease of either pathogen compared to old leaves (Sharma *et al.*, 1986).

## 7. EPIDEMIOLOGY

### a. Disease Development in Relation to Environmental Conditions

Alternaria blight of Brassicaceae caused by *A. brassicae* is favoured by low temperature, high humidity and splashing rain (Dey, 1948; Humpherson-Jones and Phelps, 1989; Louvet, 1958; Louvet and Billotte, 1964). In Canada (McDonald, 1959) and Holland (Van Schreven, 1953), optimum temperature for the development of *A. brassicae* is reported to be between 20–24°C. In India, a temperature range of 15–25°C, relative humidity of 70–90%, intermittent winter rains, and wind velocity around 2–5 Km per h has been reported to be most conducive to alternaria blight development in mustard (Ansari *et al.*, 1988b; Saharan, 1991; Saharan and Kadian, 1984; Saharan *et al.*, 1981). In France, Louvet (1958, 1963) observed severe development of *A. brassicae* at a temperature range of 15–22°C, relative humidity of 80% for a period of 36 h, and under stormy and high wind velocity conditions. Similar observations have also been made in India by Awasthi and Kolte (1989) and Chahal and Kang (1979b). Domsch (1957) reported relative humidity between 95–100% at least for 18h, and temperature of 21–27°C for three successive days as essential for epidemic development of *Alternaria* disease of rape and cabbage. *A. brassicae* forms abundant spores after a succession of wet and dry periods. The spores are probably spread over short distances by rain and wind and are dispersed over greater distances by the action of wind alone during dry weather (Louvet and Billotte, 1964). To a very limited extent slugs can spread the disease by ingesting spores which remain viable in the alimentary canal and can infect plants when excreted (Hasan and Vago, 1966).

According to Awasthi and Kolte (1989), alternaria blight of rapeseed-mustard develops best during rosette to flowering stages; relative humidity from 67–73%, rainfall >70 mm, 5–7 h of sunshine/day, and a minimum temperature range of 7–10°C concomitant with the maximum temperature range of 20–23°C have been found to be positively correlated ( $r=0.511-0.805$ ) with the severity of disease. Increase in age beyond 30 days is positively correlated ( $r=0.9802$ ) with the increase in disease severity. Similarly, Chahal (1986a) reported that the susceptibility of brown sarson to *A. brassicae* increases with the age of the host. Maximum susceptibility of rapeseed plants has been observed at 55–85 day old plants (Sarkar and Sengupta, 1978). For maximum infection and disease development in mustard a minimum period of 4 h leaf wetness is essential. Longer periods of leaf wetness at



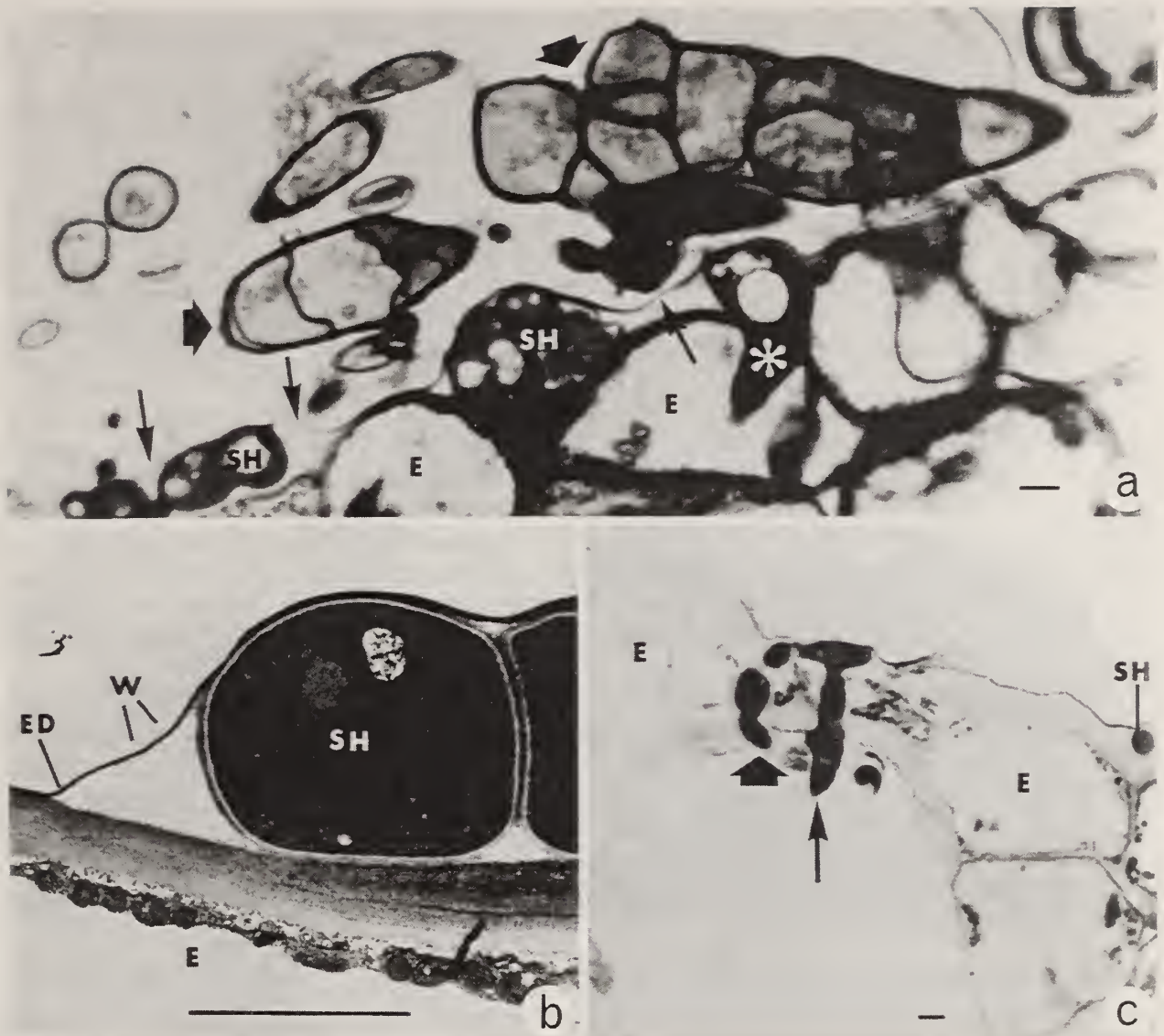


Fig. 12. *Alternaria brassicae* on the leaves of *Brassica rapa* cv. Candle. Bar = 5.0  $\mu$ m: (a) light micrographs showing the germinating conidia (broad arrows) and subcuticular hypha (SH) penetrating (asterisk) into an epidermal cell (E); narrow arrows, cuticle. x 1,200; (b) transmission electron micrograph showing subcuticular hyphae (SH), epicuticular wax layer (W), and electron-dense layer (ED) of cuticle. x 7,000; and (c) light micrograph showing a hypha penetrating through a stoma (narrow arrow) and another one penetrating into a subepidermal position (broad arrow) without becoming subcuticular. x 800 (Courtesy: Tewari, 1986).



25°C increase the infection frequency on the leaves (Kadian and Saharan, 1984; Saharan, 1991). Reduced light intensity is more favourable for lesion development. Conversely, spores produced under high light intensities show reduced germination (Kadian, 1982; Kadian and Saharan, 1984; Mukadam and Deshpande, 1979a).

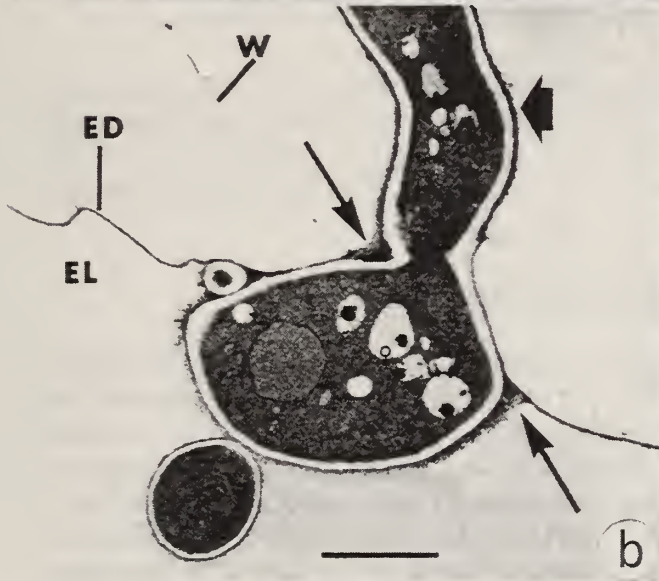
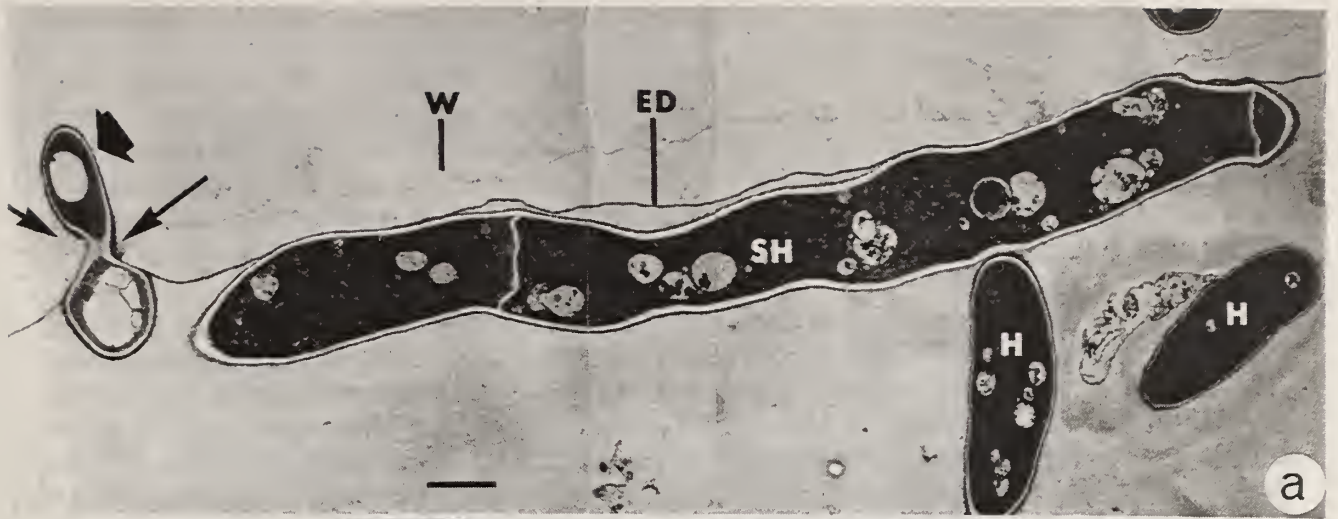
*Alternaria brassicicola* fails to produce significant infections at 10°C after 96h, while *A. brassicae* produces lesions on the host under similar conditions (Humpherson-Jones *et al.*, 1983). *A. brassicicola* lesions on overwintered leaf litter of *B. oleracea* grown for seed production, produce high concentrations of spores in the spring, and are able to initiate new infections on foliage and subsequently on inflorescence and siliquae. A vertical disease gradient develops in maturing crops, the lowest siliquae becoming infected first and infection spreading slowly upwards. Spores are produced abundantly after 20 h leaf wetness at a mean temperature of 13°C or more. Their release is stimulated by a fall in RH but inhibited at a constant high RH resulting in a daily cycle in air spore concentrations with minimum occurring in the early morning and maximum in the early afternoon (Figs. 14, 15; Humpherson-Jones and Maude, 1982a). Sporulation in *A. brassicae* and *A. brassicicola* on naturally-infected leaf discs of oilseed rape and cabbage requires RH > 91.5% and 87%, respectively. The optimum temperature for sporulation is 18–24°C for *A. brassicae*, and 20–30°C for *A. brassicicola* at which temperature both fungi produce spores in 12–14 h. Above 24°C sporulation in *A. brassicae* is inhibited. White light inhibits sporulation in *A. brassicae* with the degree of inhibition increasing with increasing light intensity (Humpherson-Jones and Phelps, 1989).

The effects of humidity, seed infection, temperature and nutrient stress on development of cabbage seedling blight caused by *A. brassicicola* under controlled environment chambers has been investigated by Bassey and Gabrielson (1983a). Cotyledon lesions are more severe with extended moist incubation following wound inoculation. The temperature of 30°C is optimum for wirestem disease from naturally-infected seed. Natural wirestem does not occur below 15°C even with a heavily infected seed lot, and little occur below 20°C with a moderately or lightly infected seed lot.

According to Singh (1988), the conidia of *Alternaria* appear in the air 8–13 days prior to the first onset of disease on the crop. The high percentage of disease incidence and disease intensity index is closely related to the high percentage of *Alternaria* spore load in the air. Maximum incidence of mustard blight depends upon high RH (77–94 %) and high temperature of 28–30°C.

*Alternaria alternata* causing leaf and pod blight of radish develops fast when mean temperature is 20.6–23.8°C and relative humidity from 57.9–67.4% (Singh and Suhag, 1983; Suhag *et al.*, 1985).

Under field conditions *A. raphani* infection progresses rapidly at 22–26°C. At high soil moisture content infection is less at 18°C (Atkinson, 1950). *Alternaria* blight development on *Crambe* is dependent on warm and damp weather (Czyzewska, 1971).





## **b. Disease Development in Relation to Nutrition and Cultural Conditions**

Closer spacing (30 x 15 cm), high doses of nitrogen (>80 Kg N/ha), and frequent irrigations are known to rapidly increase severity of alternaria blight disease of mustard (Kadian and Saharan, 1988; Saharan, 1991, 1992a; Saharan and Kadian, 1984). In Prague (Czechoslovakia) higher doses of NPK increased *A. brassicae* infection in winter rape pods. Similarly, top dressing with nitrogen in spring significantly increased *Alternaria* intensity on rape pods (Stankova, 1972).

Planting time has a major influence on the incidence of disease in mustard crops. In Haryana (India) 52% disease incidence has been recorded on mustard crop sown in the last week of October. However, when the crop is sown in the third week of November, infection fell to 15.5% (Saharan, 1984). The alternaria pod blight of radish seed crop is significantly less in the normal season transplanting of 15th December in the Punjab (India), whereas alternaria twig blight is significantly less in the early transplanting of 15th November (Sandhu *et al.*, 1984, 1985).

The dwarfing agent, Cycocel (CCC) sprayed on rapeseed crop tends to decrease infection of *A. brassicae* whereas Alar increases the infection (Strzelczyk and Rozej, 1974). The fungicide Calixin (75% w/w tridemorph) applied at 1.125 ml in 4 L water predisposes cabbage plants to *A. brassicae* and *A. brassicicola* infection (Munro, 1984).

## **c. Disease Development in Relation to Host Resistance**

*Alternaria* blight progression on highly susceptible (*B. juncea* cv. Prakash) and moderately resistant (*B. napus* cv. Tower) cultivars of rapeseed-mustard is governed by reduction in infection frequencies coupled with reduction in number and size of lesion formation due to host resistance. In highly susceptible cultivar Prakash, the onset of disease occurs earlier, with a greater abundance of large lesions. Such cultivars have a shorter latent period, abundant conidial production per lesion and higher infection rate (0.46) in comparison to moderately resistant cultivar Tower (Saharan 1991, 1992b; Saharan and Kadian, 1983). On Tower,

Fig. 13. Transmission electron micrographs of ultrathin sections of *Alternaria brassicae* on the leaves of *Brassica napus* cv. Altex. Bar = 2  $\mu$ m: (a) a penetrating germ tube (broad arrow), a subcuticular hypha (SH), and two other hyphae (H), embedded in the cell wall of the host adaxial leaf epidermal cell. Note the electron-dense material at the point of penetration (narrow arrows), the layer of epicuticular wax (W) detached from the electron-dense layer of the cuticle (ED). x 4,200; (b) a penetrating germ tube (broad arrow). Note the electron-dense material at the point of penetration (narrow arrows), the detached epicuticular wax layer (W), and electron-translucent layer (EL) of the cuticle. x 8,050; (c) junctional region between the two epidermal cells (E) showing a subcuticular hyphae (SH) and glancing section of a hypha passing through the cell wall (asterisk). The latter hypha is penetrating between the two cells. x 7,400; (d) section showing two subcuticular hyphae (SH), one of which is penetrating into the cell wall of the epidermal cell (E). x 4,200; and (e) section showing subcuticular hyphae (SH), a few other hyphae (H) in the epidermal cells, and one hypha (asterisk) below the epidermis close to the palisade mesophyll tissue. x 3,000 (Courtesy: Tewari, 1986).



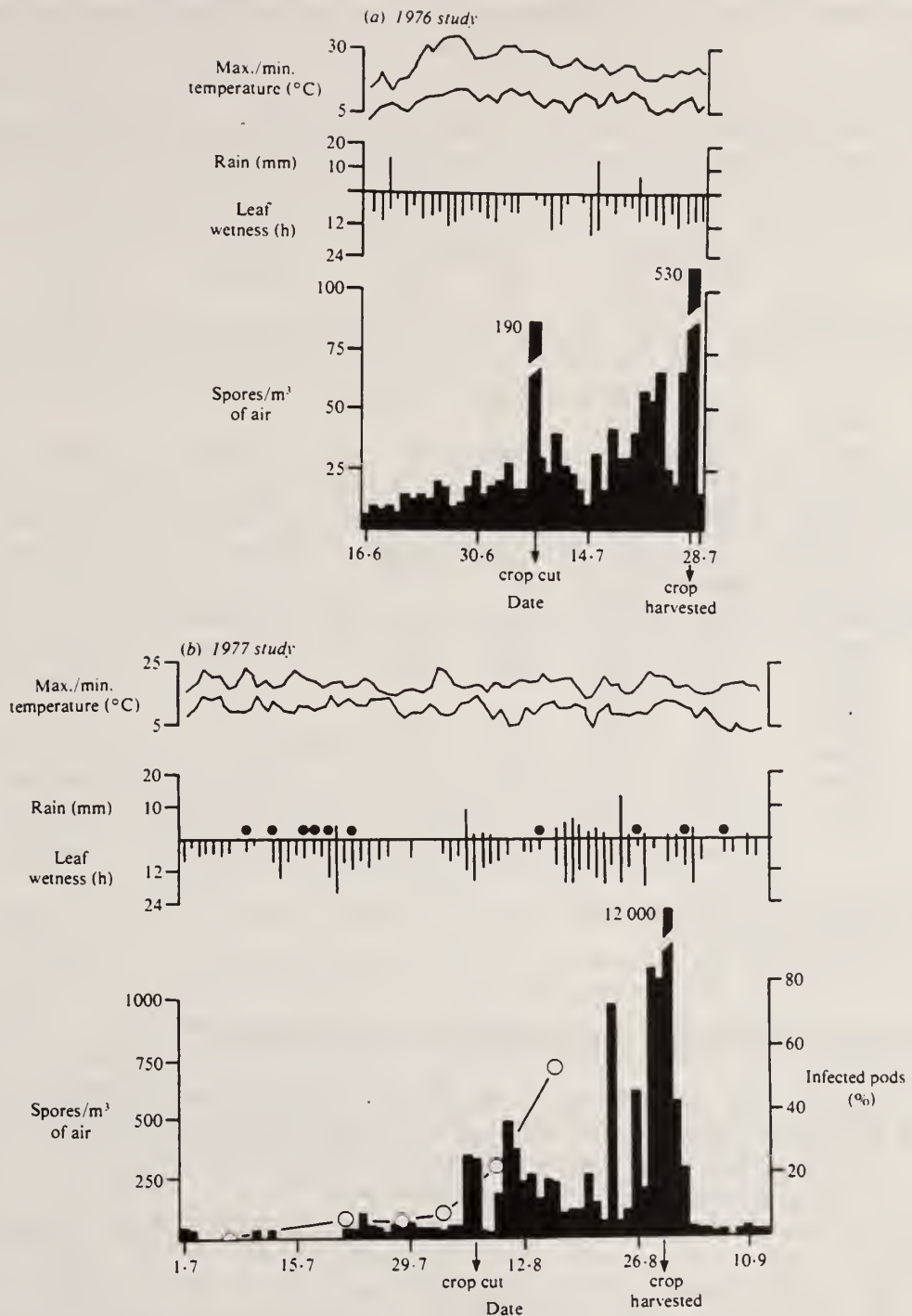


Fig. 14. The mean daily concentration of *Alternaria brassicicola* spores in the air within *Brassica oleracea* seed production crops: (a) 1976 study; and (b) 1977 study; • >0.2 mm <1 mm rain : 0 – 0 infected pods (Courtesy: Humpherson-Jones and Maude, 1982a).

infection is delayed up to 25 days with the occurrence of very few lesions of restricted size (Table 13). Tower gets 15.6% of leaf infection compared to 38.5% in Prakash.

Table 13. Factors influencing resistance/susceptibility of different cultivars of rapeseed-mustard against *Alternaria brassicae* (Saharan and Kadian, 1983)

| Cultivars | Leaf surface | Stomata <sup>1</sup> |           | Lesions/leaflet <sup>2</sup> |           | Sporulation <sup>3</sup><br>per lesion | Incubation <sup>4</sup><br>period (days) | Latent period (days) |
|-----------|--------------|----------------------|-----------|------------------------------|-----------|--|--|----------------------|
|           |              | No. (per sq. cm)     | Size (mm) | No.                          | Size (mm) |  |  |                      |
| Tower     | upper        | 145                  | 15.7      | 1.8                          | 1.9       | 80                                     | 10                                       | 16                   |
|           | lower        | 210                  | 25.9      |                              |           |  |  |                      |
| RC-781    | upper        | 166                  | 17.3      | 3.3                          | 5.7       | 92                                     | 8  | 12                   |
|           | lower        | 245                  | 26.7      |                              |           |  |  |                      |
| CSR-448   | upper        | 196                  | 19.2      | 3.7                          | 10.7      | 120                                    | 7  | 10                   |
|           | lower        | 303                  | 27.9      |                              |           |  |  |                      |
| CSR-741   | upper        | 189                  | 19.3      | 6.3                          | 14.6      | 125                                    | 7  | 9                    |
|           | lower        | 292                  | 20.0      |                              |           |  |  |                      |
| CSR-142   | upper        | 199                  | 19.3      | 4.4                          | 10.6      | 120                                    | 8  | 11                   |
|           | lower        | 306                  | 28.0      |                              |           |  |  |                      |
| YRT-3     | upper        | 225                  | 22.0      | 10.2                         | 22.3      | 250                                    | 6  | 9                    |
|           | lower        | 403                  | 30.8      |                              |           |  |  |                      |
| RH-30     | upper        | 221                  | 22.0      | 8.1                          | 19.1      | 240                                    | 6  | 9                    |
|           | lower        | 392                  | 30.6      |                              |           |  |  |                      |
| Prakash   | upper        | 229                  | 22.7      | 8.7                          | 18.9      | 260                                    | 6  | 8                    |
|           | lower        | 413                  | 31.2      |                              |           |  |  |                      |

<sup>1</sup>and <sup>3</sup> Average of 100 observations

<sup>2</sup>and <sup>4</sup> Average of 10 leaves

#### d. Models to Describe the Progress of the Disease

Several models have been proposed to describe the progress of compound interest diseases like *Alternaria* disease of crucifers (Fontem *et al.*, 1991)

1. Gompertz equations :  $Y = - \ln [-\ln(y)]$

2. Logisitic equations :  $Y = \ln [y/(1-y)]$

These equations were compared by Berger (1981) to describe plant disease progress.

3. Weibull function :  $Y = \{ \ln[1/(1-y)] \}^{1/c}$

It has received much attention recently because of its flexibility and simplicity (Fontem, 1986).

4. Gregory power function:  $y = ax^b$ , in which  $y$  is the proportion of disease at  $x$  units of distance from the source,  $a$  is the value of  $y$  at  $x=1$ , and  $b$  is the rate of change in  $y$  with change in  $x$ ; i.e.,  $b$  is an estimate of the slope of the gradient and the value of  $b$  is usually negative. The transformation equation is  $\log(y) = \log(a) + b \log(x)$ . Since this model cannot be used to predict disease at the inoculum source (distance  $x = 0$ ), Gregory's model was modified by Mundt and Leonard (1985) to

include a translation factor (m) [ $y=a(x+m)^b$ ], which allows the estimation of disease at the source when  $x=0$ . Values of m are approximately the radii of the sources.

5. Kiyosawa and Shiyomi (1972) model:  $[y=a \exp(-bx)]$ .

This model has been used to describe spore dispersal in cvs with multilines.

6. Lambert *et al.* (1980) model:  $[y=a \exp(ax^n)]$  with mixed power and exponential terms and a shape parameter n.

7. Maffia (1985) hollow shaped curves:  $[y=ax^b \exp(nx)]$ . Sometimes  $\ln[y/(1-y)]$  or  $-\ln[-\ln(y)]$  is plotted over  $\log_{10}(x)$  or x to linearize hollow-shaped curves.

To illustrate some possibilities, Fontem *et al.* (1991), evaluated progress and spread of dark leaf spots in three cabbage cultivars during two seasons by fitting representative curves of eight gradient models (Fig. 16).

Three growth models (logistic, Gompertz, and Weibull) are fitted to the severity values by nonlinear regression. The shape parameter (c) for the Weibull function average 3.61. The average daily epidemic rates with the logistic model are  $K=0.06$  in the winter and  $k=0.11$  in the spring. Final disease severity (yt) at the source average 0.52 in the winter and 0.97 in the spring. The nearly flat gradients from 1 to 6.7 m are fitted satisfactorily by each of above mentioned gradient models, but the total gradient is described adequately only by the modified model of Gregory and the Hoerl function.

The three models are modified as:

1. Logistic (Berger, 1981):  $Y_t = 1/\{1+\exp[a+k_1t]\}$
2. Gompertz (Berger, 1981):  $Y_t = \exp[-a \exp(-Kgt)]$
3. Weibull (Pennypacker *et al.*, 1980):  $Y_t = 1-\exp\{-Kw(t-a)\}^c$

In these models,  $Y_t$  is the disease proportion at time t, and k is the rate parameter. The term "a" in the three models is a parameter for initial disease; in the logistic model,  $a = -\ln[Y_0/(1-Y_0)]$  and in the Gompertz model,  $a = -\ln(Y_0)$ . In the Weibull function, a positions the curve on the time axis, and c is the curve-shape parameter. In these analyses,  $Y_0$  is the amount of disease at first observations, and the maximum amount of possible disease ( $Y_{max}$ ) is assumed to be 1.0. The  $R^2$  and the residual sum of squares are used to evaluate the magnitude of variation among the data that is explained by the model (goodness of fit) (Cornell and Berger, 1987; Daniel and Wood, 1980). The pattern of scatter when residuals ( $Y$  observed -  $Y$  predicted) are plotted vs. time is used to confirm the suitability of the model (Cornell and Berger, 1987). The progress of disease among cultivars is compared with observed initial disease ( $Y_0$ ), epidemic rate (k), final disease severity ( $Y_f$ ), and the area under the disease progress curve (AUDPC) (Shaner and Finney, 1977).

Similar statistical methods are used to evaluate models for the spread of disease. A non linear curve fitting program (Berger, 1988) is used to evaluate five gradient models:

1. Gregory (1968):  $y=ax^b$
2. Modified Gregory (Mundt and Leonard, 1985):  $y=a(x+m)^b$



3. Kiyosawa and Shiyomi (1972) :  $y = a \exp(-bx)$
4. Lambert *et al.* (1980) :  $y = a \exp(-bx^n)$
5. Hoerl (Daniel and Wood, 1980; Maffia, 1985):  $y = ax^b \exp(nx)$

In these models,  $Y$  is the proportion of disease severity at  $x$  units of distance from the inoculum source and  $b$  is the slope parameter. In the model of Gregory (1968),  $a$  is the disease proportion at one unit of distance from the source, whereas in the modified Gregory (Mundt and Leonard, 1985) model  $a$  is the disease proportions at 1-m units of distance. In the models of Lambert *et al.* (1980), and Hoerl (Daniel and Wood, 1980),  $n$  is a parameter that modifies the curve shape, and this parameter increases the flexibility and applicability of the models if curve shapes vary.

Three other gradient models were examined in their linearized form because the models contained logarithms both to base  $e$  ( $\ln$ ) and to the base 10 ( $\log$ ), and the non-linear equations were rather complex.

1. Logistic gradient model (Berger and Luke, 1979):  $\ln[y/(1-y)] = \ln[a/(1-a)] - b \log(x)$
2. Gompertz gradient model (Danos *et al.*, 1984):  $-\ln[-\ln(y)] = \ln[-\ln(a)] - b \log(x)$
3. A variant of the logistic gradient model (Minogue and Fry, 1983):  $\ln[y/(1-y)] = \ln[a/(1-a)] - bx$ .

In these regressions,  $a$  is the average proportion of disease on the plants closest (0.3 m) to the inoculum source. These transformations are compared by the  $R^2$  and for significance of the slope of the regression by  $t$  test.

The volume under the disease progress surface (VUDPS), as suggested by Maffia (1985), and the isopathic rate (rate of movement in space of a given level of disease severity) (Berger and Luke, 1979) are calculated from the progress and spread characteristics of the disease. The VUDPS is calculated from the AUDPC at each point in space as:  $VUDPS = \sum [(A_i + 1 + A_{i+1})/2][X_{i+1} - X_i]$

Where  $A_i$  = the AUDPC at  $X_i$  units of distance from the source. The isopathic rates are calculated from the source to 6.7 m for the  $y=0.1$  isopath in the winter season and  $y=0.05$  isopath in the spring season.

## 8. FINE STRUCTURES AND ELECTRON MICROSCOPY

The sequence of events in the spore production and maturation of *A. brassicicola* was described by Campbell (1964, 1969a,b, 1970a,b, 1972). Each cell of the mature spore of *A. brassicicola* has a two-layered wall, the layer distant from the protoplast being melanized. The mature septa are five-layered, having two layers of secondary wall on either side of the septal partition which is itself three-layered. Each septum has one simple pore. New spores are produced by an outgrowth, through a pore, of the inner wall layer of the mother cell. Young spores have many small mitochondria and much vesicular endoplasmic reticulum, and as they mature, lipid bodies and an unknown polyglucoside are produced. Mature

spores have glycogen but very little, if any, lipid. The vesicular endoplasmic reticulum, multivesicular bodies and lomasomes are involved in wall formation. The plasmalemma has rectangular grooves in its outer surface and corresponding ridges on the inner one; both surfaces bear particles of two distinct sizes. The endoplasmic reticulum may be lamellated or vesicular and is involved in wall formation. The vesicles produced by the endoplasmic reticulum fuse with the plasmalemma. However, dormant spores (Campbell, 1970b) have very thick, heavily pigmented, melanized walls with plugged septal pores. The small amounts of endoplasmic reticulum and the few mitochondria lie near the plasmalemma. The germ-tube walls arise from the inner layers of the spore walls. Lomasomes and endoplasmic reticulum vesicles are probably concerned with this wall formation. During germination, mitochondria and ribosomes increase in numbers, first in the germinating cell and then in the germ tube. As the hyphae age they produce lipid droplets and vacuoles, the latter finally fill most of the cell as the cytoplasm degenerates. Conidiophores have a similar structure to mature hyphae, except that after spore production, they have a pore in the tip and an annulus. In an albino strain of *A. brassicicola* (Campbell *et al.*, 1968), when compared with wild type (Campbell, 1969a,b, 1970a), there was little difference in the structure of the hyphae and the conidiophores except that there were no electron dense deposits of melanin in the wall, and the pore in the conidiophore had no annulus (Campbell, 1970b).

Giemsa stained preparations of *A. brassicicola* show variations in the number of nuclei in the cells of the vegetative hyphae from one to many, with the hypha tip cells having up to 27 nuclei and older cells up to 33. There are fewer nuclei (0-3) in the cells of mature conidiophores, and one or two nuclei in most cells of the conidia in three-day old cultures grown on potato carrot agar. Nuclei are also seen wedged in the connections between conidia and in the basal apical pores of conidia after separation. They are less frequently wedged in septal pores in the conidia. Six chromosomes are present in dividing nuclei in the vegetative hyphae (Figs. 17, 18; Knox-Davies, 1979).

## 9. BIOCHEMISTRY

### a. Biochemical Changes in the Host

The effect of *A. brassicicola* infection on the changes of nucleic acids of *B. oleracea* var. *botrytis* has been studied by Maitra and Samajpati (1985). An increase in total nucleic acids (97%), DNA (44%) and RNA (150%) in infected leaves is recorded. The increase in RNA content is due to an increase of each of the four nucleotides. However, guanylic acid increases significantly more than the other three nucleotides. The increase in G/C ratio is also due to an increase in guanylic acid.

Losses of soluble carbohydrates are high from dead or damaged seeds, moderate from immature seeds or seeds which germinate slowly, and low from mature seeds with dark, leathery testas which germinate normally. Loss of electrolytes is higher from dead, damaged, immature seeds and seeds which



germinate slowly than from mature seeds which germinate normally (Knox-Davies, 1980).

Green islands were observed around infected spots of *A. brassicicola* and *A. brassicae* on mustard leaves by Mandahar and Suri (1983, 1987). These green islands have higher content of cytokinin-like substances, compared to the surrounding yellowed and healthy tissues. The presence of starch in green islands is correlated with formation of metabolic sinks because of their higher content of cytokinin-like substances (Suri *et al.*, 1983). Cytokinin-like substances appear to be actively involved in infection and pathogenesis of *A. brassicicola* (Suri and Mandahar, 1984, 1985, 1986). Production of pectolytic and cellulytic enzymes by *A. brassicae* causing leaf spot of *B. rapa* has been observed (Srivastava and Srivastava, 1982; Suri and Mandahar, 1982). *A. brassicae* produces invertase and amylase on cabbage decoctions (Weimer, 1924). *A. brassicae* infection in cauliflower leaves brings changes in polyphenoloxidase and peroxidase (Maitra and Samajpati, 1982). The presence of large number of enzymes viz., amylase, invertase, pectinmethyl esterase, phosphorylase, aldolase, amidases, ribonuclease, alkaline phosphorylase, nucleophosphatases, catalase, dehydrogenases, deaminases, nucleodeaminases, glycerophosphatase, cellulase and B-glucosidase in *A. alternata*, *A. brassicicola* and *A. raphani* infecting crucifers has been demonstrated (Desgupta and Verma, 1961, 1962; Verma, 1964, 1971).

*Alternaria brassicicola* stimulates ethylene production in closed culture with floating leaf discs from cabbage. Production is increased by preculturing the fungus on media containing cabbage components, but which contains little or no methionine. It suggests that the nature of parasitism of this pathogen on cabbage is characterized by a latent capability to cause the production of the plant senescing hormone ethylene (Poapst *et al.*, 1979).

#### **b. Biochemical Changes in the Pathogen**

*Alternaria brassicae* and *A. brassicicola* produce antibiotics which are active against bacteria, fungi and algae (Lindenfelser and Ciegler, 1969). *A. brassicicola* strain 13 is reported to form extracellular lipids like phospholipids, mono- and di-glycerides, sterines, free fatty acids (predominant) and triglycerides (Aizina, 1977). The biosynthesis of lipids and phospholipids is highest on Czapek medium (Aizina *et al.*, 1976).

A melanoid pigment insoluble in strong alkali has been isolated from *A. brassicae* mycelium. Melanins are important for survival and longevity of fungal propagules. Abscissic acid (ABA), a naturally occurring plant growth regulator involved in the control of various plant processes, also has been characterized in the mycelia of the black spot fungus *A. brassicae* (Dahiya, 1988; Dahiya *et al.*, 1988).

The germinating spores of *A. brassicae* and *A. brassicicola* isolated from mustard are able to hydrolyse the various carbohydrates which are used in germination media. *A. brassicae* has a greater ability to hydrolyze pectin and carboxymethyl cellulose than *A. brassicicola*, whereas the reverse is true for the hydrolysis of sodium polypectate (Mukadam and Deshpande, 1979b).



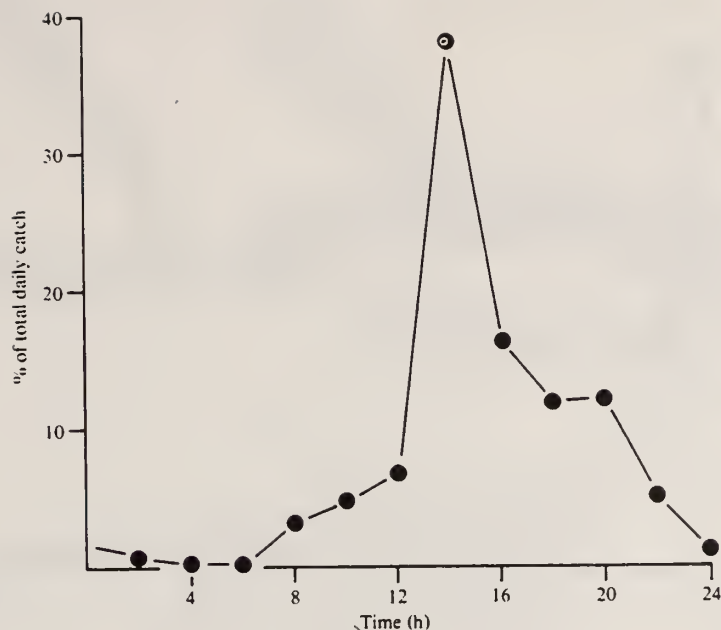


Fig. 15. The hourly concentration of *Alternaria brassicicola* spores in the air within a cabbage seed crop. Mean values of seven high spore count days (Courtesy: Humpherson-Jones and Maude, 1982a).

The composition of amino acids extracted from *A. brassicicola* and *A. brassicae* is only slightly different from those of *A. raphani*. This similarity of amino acids in the first two pathogen extracts is correlated with the severe to moderate susceptibility of the varieties of crucifers, and the difference of amino acids contained in *A. raphani* extract may have accounted for the severe susceptibility only on radish. One amino acid of Rf 0.66 of *A. raphani* extract corresponds to that of the radish extract at about the same level (Changsri, 1961).

The conidia of *A. brassicicola* are remarkably resistant to phytoalexins such as phaseollins, phaseollidin and phaseollinisoflavan, often germinating in assays using 100 µg/ml (Skipp and Bailey, 1977). Similarly, 3-day-old spores were able to grow in relatively high levels of these phytoalexins.

Dahiya and Tewari (1991) identified three plant growth factors from *A. brassicae*, and treatment of seedlings of canola/rapeseed with two of them, N-Methyl-2, 5-dimethyl-N'-cinnamoylpiperazine and 3-carboxy-2-methylene-4-pentenyl-4-butenolide, reduced their growth whereas 2-hydroxy-1'-methylzeatin increased the growth of seedlings.

### c. Glucosinolates

Glucosinolates occur as glucosides throughout the Brassicaceae. They are present in the seed and other parts of oilseed rape. The hydrolysis products of gluco-

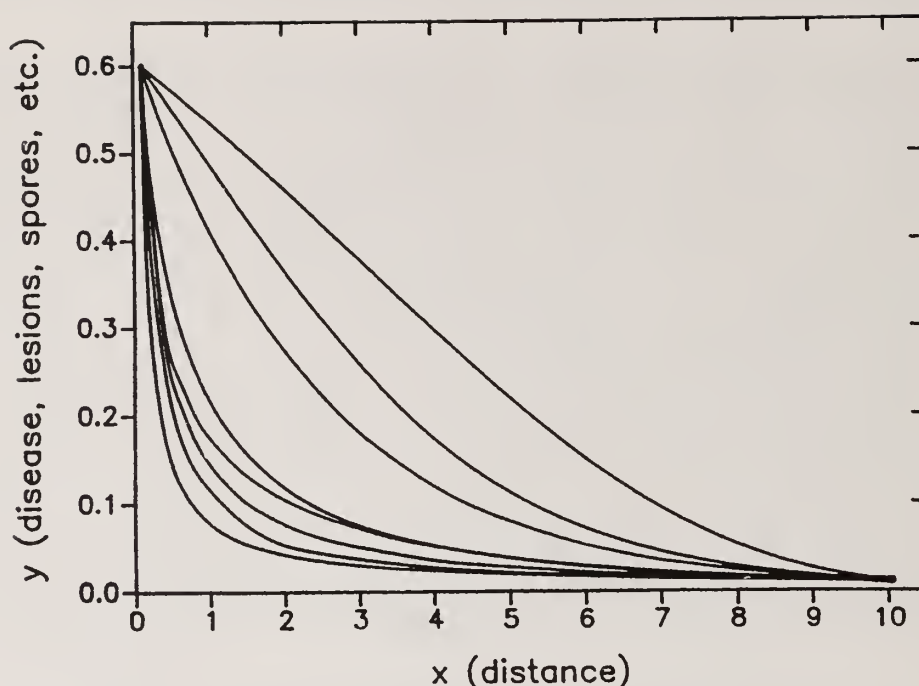


Fig. 16. Representative curves of eight gradient models. The curves from the bottom to the top of the figure at  $x = 1.0$  were generated by the following models:  $y = ax^b$  (Gregory);  $\ln [y/(1-y)]$  vs.  $\log (x)$ ;  $y = a \exp (bx^n)$ ,  $n = 0.2$  (Lambert *et al.*);  $y = ax^b \exp (nx)$ ,  $n = -0.2$  (Hoerl);  $-\ln [-\ln(y)]$  vs.  $\log (x)$ ;  $y = a \exp (-bx)$  (Kiyosawa and Shiyomi);  $\ln [y/(1-y)]$  vs.  $x$ ; and  $-\ln [-\ln (y)]$  vs.  $x$ . All curves began with proportion  $y = 0.6$  at distance  $x = 0.1$  and decreased to  $y = 0.01$  at  $x = 10.0$ . Different curve shapes are possible with the models of Lambert *et al.*, and Hoerl by using other values for the shape parameter ( $n$ ). The curve shown for the Gregory model typifies many of the steep gradients observed for *Alternaria brassicicola* on cabbage in which  $y$  = disease proportion and  $x$  = meters (Courtesy: Fontem, Berger, Weingartner and Bartz, 1991).

sinolates include nitriles, isothiocyanates, oxazolidinethiones and thiocyanates which can be unpalatable and toxic to non-ruminant animals. Thus, the presence of glucosinolates in rape meal has limited its use in animal feed, necessitating the development of the so called "double-low" lines. These produce seed low in erucic acid and glucosinolates. A decreased content of glucosinolates in oilseed rape tissues may have negative consequences for pest and disease incidence on the crop. The hydrolyzed products of glucosinolates are fungitoxic to rapeseed pathogens *in vitro* (Mithen *et al.*, 1986) and there is also much indirect evidence to suggest that their presence contribute to resistance to a range of oilseed rape pathogens (Greenhalgh and Mitchell, 1976; Mithen *et al.*, 1987; Rawlinson *et al.*, 1985). Doughty *et al.*, (1991) examined the changes in the glucosinolate content of leaves following inoculation with *A. brassicae* in cultivars Bienvenu (single-low) and Cobra (double-

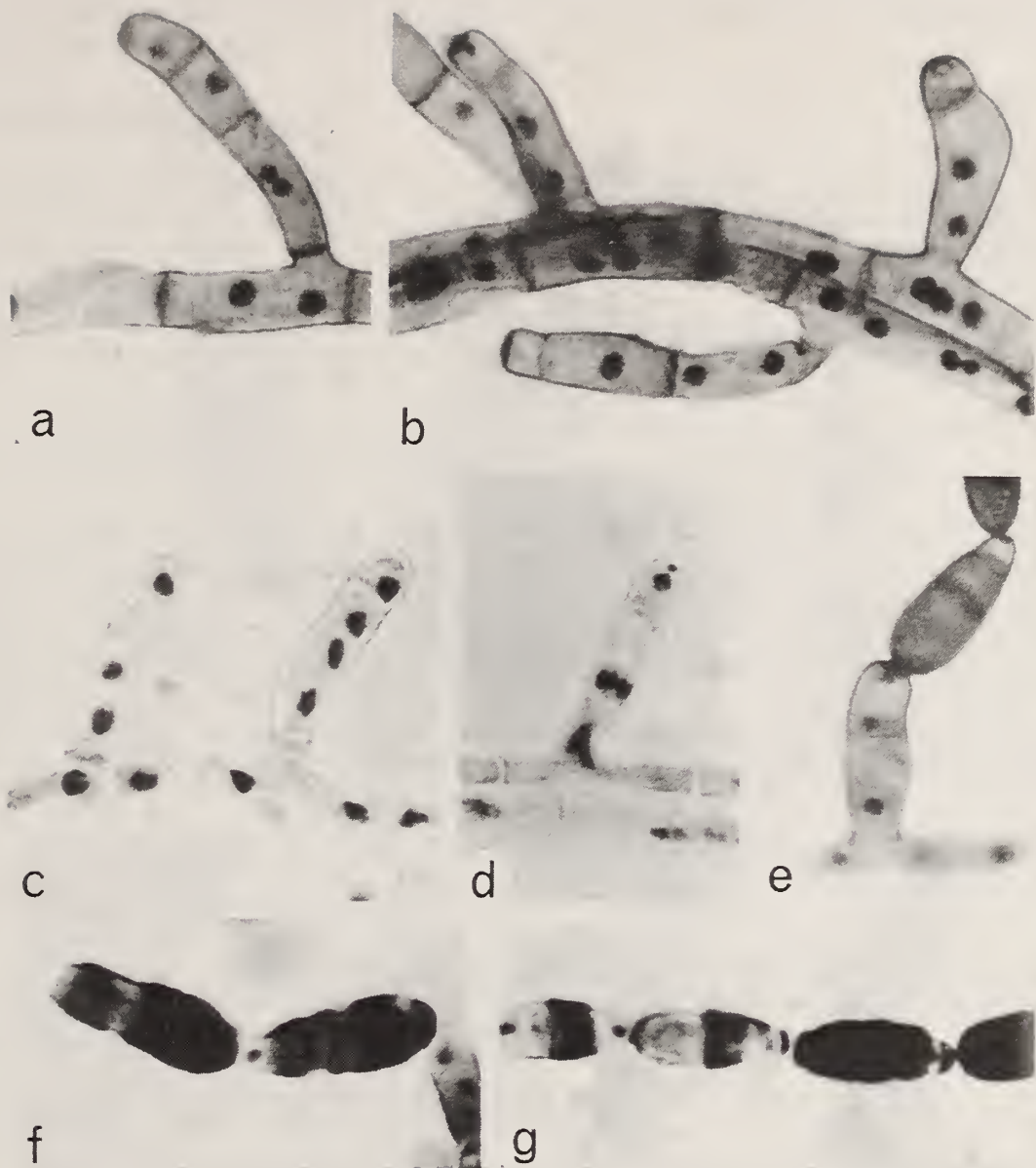


Fig. 17. Conidiophores of *Alternaria brassicicola* showing (a) uni- and binucleate cells and apical cell with pore and annulus; (b) typical complement of nuclei in basal cells, anucleate terminal cells (right and bottom), pore and annulus (right), and nuclear material and cytoplasm wedged in pore in terminal cell (left); (c) typical complement of nuclei in subtending, basal and terminal cells; (d) nucleus wedged in pore of terminal cell; (e) developing conidia (only one shown) with nucleus wedged in pore between terminal cell of conidiophore and basal cell of developing conidium; and (f, g) conidia with thick, roughened wall material which stained intensely with Giemsa (Courtesy: Knox-Davies, 1979).



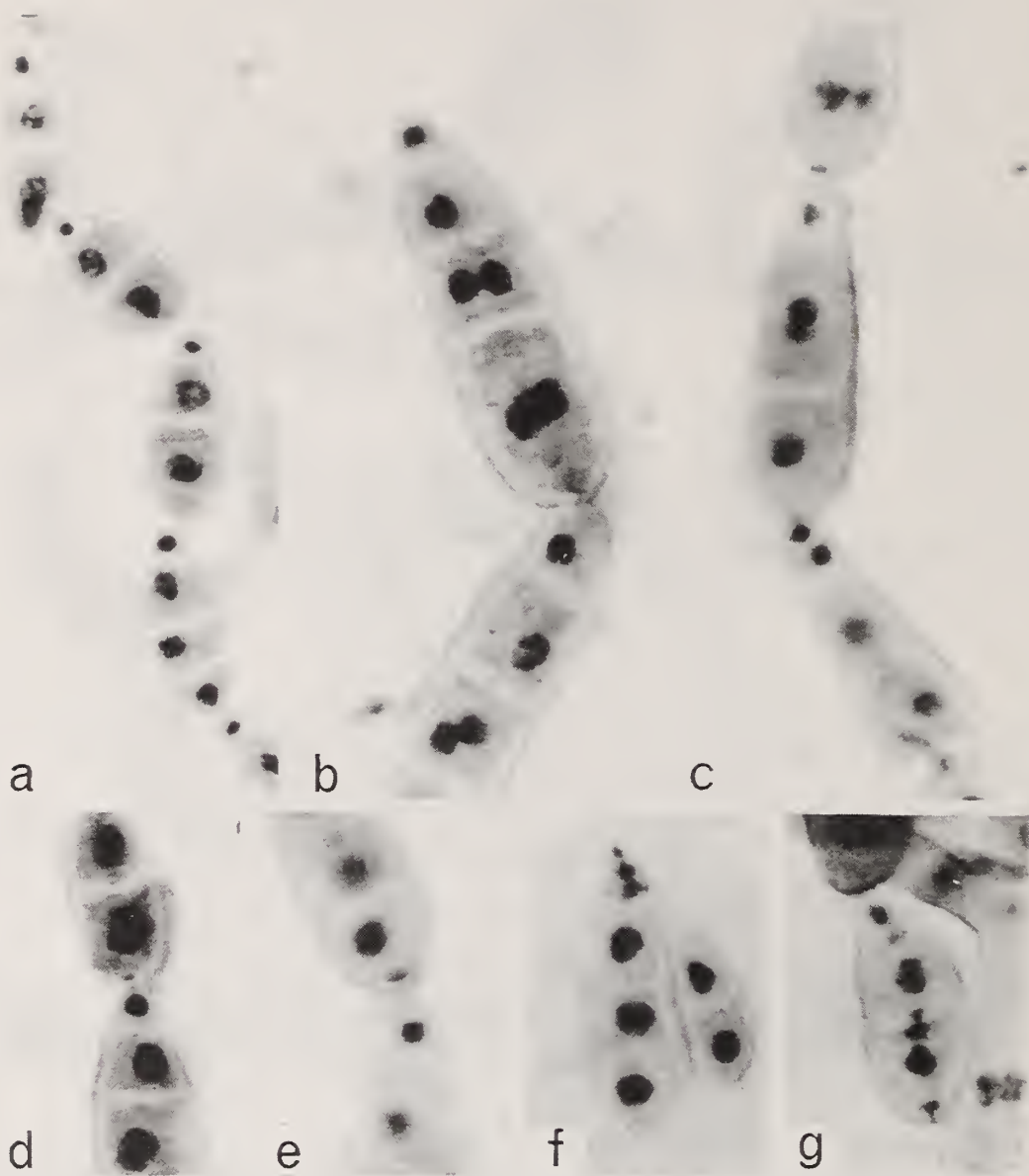


Fig. 18. Conidia of *Alternaria brassicicola* showing: (a) small, intensely staining nuclei of the terminal cells and the wide pores of the basal cells; (b) septal pores, typical distribution of nuclei and small, intensely staining nucleus of the terminal cell; (c) Giemsa-stained material wedged in septal pores between adjacent conidia and in a septal pore within a conidium; (d, e) Giemsa-stained material wedged in the septal pores between adjacent conidia in a conidial chain; (f) Giemsa-stained material wedged in the terminal pore of a conidium; and (g) Giemsa-stained material wedged in basal and interstitial pores of a conidium (Courtesy: Knox-Davies, 1979).

low). The glucosinolate content of leaves increased markedly after inoculation, but the response depended on leaf age and cultivar. Glucosinolates accumulated more in the sixth leaf of Bienvenu.

#### **d. Metabolites Produced**

*Alternaria brassicae* when grown in liquid still culture produces host specific phytotoxins which have been shown to be cyclodepsipeptides. It also produces several other metabolites including the new drimane sesquiterpenes deoxyuvidin B, albrassitriol, and isoalbrassitriol, as well as brassicadiol, a C<sub>15</sub> prenylated pentaketide. These compounds show no phytotoxicity on canola (Ayer *et al.*, 1987; Ayer and Pena-Rodriguez, 1987a, 1987b).

The earliest reports on phytotoxin production by *A. brassicae* or *A. raphani* are those of Degenhardt (1973, 1978) and Husain and Thakur (1966).

Cytotoxicity tests with extracts of culture filtrates of *A. brassicae* and *A. brassicicola* show that both species produce compounds which are toxic to human epithelial (HEP2) cells but there is no clear relationship between *Alternaria* infection of seed and its cytotoxicity. Metabolites produced by the two fungi are also phytotoxic, giving rise to necrosis of leaf discs and inhibition of seedling root growth, but extracts of the less specialised parasite, *A. alternata*, cause more severe phytotoxic effects on crucifer test plants. Extracts of culture filtrates from *A. brassicae* and *A. brassicicola* are found to show non-specific phytotoxicity (McKenzie *et al.*, 1988).

### **10. RESISTANCE**

Resistance to *Alternaria* species in the Brassicaceae has many components. The information on the nature and mechanism of host resistance against this pathogen is rather limited.

#### **a. Genetics of Host-Parasite Interaction**

In *B. juncea* cultivar RC781 resistance to *A. brassicae* is reported to be governed by a single dominant gene (Tripathi *et al.*, 1980). However, Brun *et al.*, (1987a,b, 1989) reported a high degree of resistance in *Sinapis alba* cultivar Emergo against *A. brassicae*. Saharan and Kadian (1983) analysed different components of horizontal resistance (HR) in *Brassica* genotypes against *A. brassicae* and found large differences in the number of lesions, size of lesions, latent period, sporulation capability and infection rate in *B. napus* cultivar Tower and in *B. juncea* cultivar RC781. In other *B. juncea* genotypes like CSR142, and CSR448 moderate level of HR has been recorded. In Tower, infection is delayed up to 25 days with very few lesions (0.95) of restricted size. Both Tower and RC781 have longer latent periods of 18 and 12 days, respectively, compared to 3 days in Prakash. The amount of conidial production is higher (265 conidia per lesion) in Prakash than (92 conidia per lesion) in Tower (Saharan, 1991, 1992b). Kolte (1987) suggested that size of lesions and amount of sporulation may be considered as important factors in

determining the degree of resistance in *Brassica* species to alternaria blight disease.

### **b. Epicuticular Wax**

The structure of wax in *Brassica* spp. and its role in host resistance has been studied by a number of workers (Conn and Tewari, 1989a,b; Holloway *et al.*, 1977; Saharan, 1992b; Skoropad and Tewari, 1977; Tewari, 1991a; Tewari and Skoropad, 1976; ). In the canola type cultivars Candle, Tobin and Altex, the wax is organized into an amorphous layer on which is situated a crystalline layer. The crystalline layer consists of plate-like crystals and a layer of erect filamentous and rod-like crystals (Figs. 19, 20, 21; Conn and Tewari, 1985, 1989a,b). The wax in *Brassica* spp. is complex both structurally and chemically (Conn, 1986; Conn *et al.*, 1984; Holloway *et al.*, 1977). In the canola-type cultivars, the wax has the same nine major constituents (alkanes, esters, ketones, aldehydes, secondary alcohols, ketols, primary alcohols, triterpenols, and fatty acids) but in varying proportions (Conn, 1986; Conn *et al.*, 1984; Tewari, 1991a,b). In the *B. napus* ssp. *oleifera* cultivar, Altex the major constituents of wax include C<sub>29</sub> alkanes, C<sub>29</sub> ketone, C<sub>29</sub> secondary alcohol and C<sub>40</sub> - C<sub>48</sub> esters (Conn, 1986; Tewari, 1991a,b).

In providing resistance to the host against *Alternaria* spp. wax appears to present only a physical barrier without a direct chemical effect (Conn, 1986; Conn and Tewari, 1989a,b; Skoropad and Tewari, 1977; Tewari and Skoropad, 1976). The wax forms a hydrophobic coating and reduces the deposition of water-borne inoculum. Wax also reduces the rate of conidium germination and the number of germ tubes formed by each conidium. The crystalline wax layer is made fluffy by enclosed air pockets which may be responsible for the above two effects by impeding the movement of plant exudates. Plants of *B. napus* ssp. *oleifera* are very waxy compared to those of *B. rapa* ssp. *oleifera* which is more susceptible to *A. brassicae*. The leaves of cultivars Midas and Tower (*B. napus*), resistant to *Alternaria*, have appreciable amounts of epicuticular wax (Skoropad and Tewari, 1977). According to Gupta *et al.* (1987a,b,c), *Alternaria* resistant genotypes viz., Tower, HNS-3 (*B. napus*), HC-2 (*B. carinata*) and *B. alba* have higher amounts of wax on their leaves at different growth stages as compared to susceptible genotypes BSH-1 and YSPB-24 (*B. rapa*) and RH-30 (*B. juncea*).

### **c. Biochemical Basis of Resistance**

The *Alternaria*-resistant *B. juncea* genotype RC781 is characterized by relatively high concentrations of phenols and low concentrations of sugars and nitrogen compared to a susceptible genotype Prakash (Gupta *et al.*, 1984). The leaf surface constituents viz., wax, total phenols, soluble nitrogen, total soluble and reducing sugars of susceptible genotypes BSH-1, YSPB-24 (*B. rapa*), RH-30 (*B. juncea*) and resistant genotypes Tower, HNS-3 (*B. napus*), AC-2 (*B. carinata*) and local (*B. alba*) have been determined by Gupta *et al.* (1987a,b,c), at 30, 50, 70, and 90 days of growth. The concentration of the phenolic compounds is remarkably higher in resistant species compared to susceptible ones at all stages of plant growth. Total soluble sugars, reducing sugars and soluble nitrogen levels are, however, lower in resistant genotypes.



#### d. Elicitation of Phytoalexins

The accumulation of phytoalexins in brassicas after exposure to microorganisms and their role in disease resistance have been demonstrated by many workers. They are low molecular-weight, antimicrobial compounds that are both synthesized by, and accumulated in plants after exposure to microorganisms (Paxton, 1981). So far, approximately a dozen different phytoalexins have been reported from crucifers (Bains and Tewari, 1985; Browne *et al.*, 1991; Devys *et al.*, 1988; Takasugi *et al.*, 1988). All phytoalexins identified from brassicas contain sulphur in their molecules.



Fig. 19. Scanning electron micrograph of air-dried, osmium vapor-fixed, and gold-coated middle leaves of *Brassica rapa* cv. Candle showing wax crystals. Bar = 2  $\mu$ m (Courtesy: Tewari, 1991).

Conn *et al.* (1987, 1991) and Tewari *et al.* (1987, 1988) described elicitation of phytoalexins in *B. rapa* var. *oleifera* (canola), *B. rapa* ssp. *rapifera* (an accession of turnip), *B. napus*, *Camelina sativa*, *E. sativa* and *C. bursa-pastoris* similar to that of cyclobrassinin. However, the quantity produced in turnip is much greater. Jejelowo *et al.* (1991) studied the kinetics of phytoalexin elicitation in *C. sativa*. An accession of *E. sativa* is reported to show hypersensitive response to *A. brassicae* (Conn and Tewari, 1986). Browne *et al.* (1991) described camalexin and methoxycamalexin phytoalexins from *C. sativa* which are thiazoyl-substituted indole phytoalexins and show strong structural resemblance to the fungicide thiabendazole.

The relationship between conidial concentration, germling growth and phytoalexin production by *C. sativa* leaves inoculated with *A. brassicae* has been studied by Jejelowo *et al.* (1991). The rapid rate of phytoalexin accumulation shortly after inoculation results into inhibition of fungal growth on the leaf surface. The phytoalexin slows germination and inhibits germ-tube growth of *A. brassicae* conidia *in vitro*.



Fig. 20. Scanning electron micrograph of air-dried, osmium vapor-fixed, and gold-coated stem of *Brassica rapa* cv. Tobin showing flat and erect wax crystals. Bar = 1  $\mu$ m (Courtesy: Tewari, 1991).

*Capsella bursa-pastoris* shares one phytoalexin with *C. sativa* and the other one with rapeseed and is also highly resistant to *A. brassicae* (Conn *et al.*, 1988). The highly resistant *C. sativa* elicits large quantities of two novel phytoalexins. It appears that in crucifers, both the quantity and quality of phytoalexins are important in regulating resistance to *A. brassicae*. These phytoalexins are highly antimicrobial, as shown by the *Cladosporium* sp. thin-layer chromatography bioassay (Tewari, 1991a,b).

Antifungal compounds have been found to accumulate when root slices of radish are inoculated with the spore suspension of *A. alternata*. Antifungal activity of the diffusate shows inhibitory action to spore germination, germ tube elongation and radial mycelial growth. The phytoalexins produced are fungistatic in nature (Kulshreshtha and Chauhan, 1985).

#### e. Calcium Sequestration

The plant cell walls are rich in calcium which is tightly bound to pectins. Calcium is known to be a factor in disease resistance. The insoluble calcium polypectates are resistant to hydrolysis by pectolytic enzymes produced by the pathogens (Vidhyasekaran, 1988). Oxalic acid and possibly other organic acids produced by various pathogens sequester calcium, which may overcome this form of resistance (Punja and Jankins, 1984; Rao and Tewari, 1987). Examination of blackspot lesions on rapeseed leaves by scanning electron microscopy in conjunction with energy-dispersive X-ray microanalysis has revealed sequestration of calcium by *A. brassicae*. Therefore, there are possibilities of enhancing resistance to *A. brassicae* in rapeseed by soil or foliar application of calcium compounds (Tewari, 1991a,b). Foliar sprays of calcium compounds sequester the organic acids at the site of infection, and soil application has the potential of boosting calcium content of the plant.



Table 14. Brassica germplasm holdings at different organizations and research centres in the world

| Name of organization<br>/ research centre   | Germplasm<br>accessions<br>(total number)          | References                                  |
|---|--|---|
| 1. The National Bureau of<br>Plant Genetic Resources<br>(NBPGR), New Delhi, India   | 3013   | Paroda <i>et al.</i> , 1987;<br>Arora, 1988 |
| 2. ICAR Coordinated Project<br>Centres on rapeseed-<br>mustard, Project Coordinator<br>unit, Hisar, Haryana, India                  | 16116  | AICORPO, 1989;<br>Kumar and Kumar,<br>1989  |
| 3. Plant Genetic Resources<br>Centre, Addis Ababa,<br>Ethiopia (PGRC/E)   | 668  | Worede, 1987                                |
| 4. National Institute of<br>Horticultural Research<br>Wellesbourne, Warwick, U.K.   | Large collection<br>exists                         | Nuej <i>et al.</i> , 1987                   |
| 5. Biotechnology Department,<br>Polytechnic University,<br>Valencia, Caminode, Spain  | 150  | Nuej <i>et al.</i> , 1987                   |
| 6. The Crucifer Genetics<br>Cooperation (CrGC),<br>Department of Plant<br>Pathology, University<br>of Wisconsin, Madison,<br>U.S.A. | 70 X 17 stocks<br>in each <i>Brassica</i><br>crop  | Williams, 1988 b                            |
| 7. Wisconsin fast plant<br>(WFP), Department of<br>Plant Pathology ,<br>University of Wisconsin,<br>Madison, U.S.A.                 | Rapid cycling<br>of <i>Brassica</i><br>seed stocks | Williams, 1988 a                            |
| 8. The Asian Vegetable<br>Research and Development<br>Centre (AVRDC), Shanhua,<br>Taiwan  | 822  | Anonymous, 1984;<br>Opena and Lo, 1981      |

#### f. Sources of Resistance

Digenomic *Brassica* species such as *B. napus* and *B. juncea* have better sources of resistance than the monogenomic (Tables 14, 15) species like *B. rapa*. Some lines of *B. alba*, *B. carinata*, *C. sativa*, *C. bursa-pastoris* and *N. paniculata* have shown resistance to *A. brassicae* in different areas of the world (Brun *et al.*,



Table 15. Sources of resistance to *Alternaria brassicae* and *Alternaria brassicicola*

| Host species/ genotype  | Common name           | References   |
|---|-----------------------|--|
| <b><i>A. brassicae</i></b>  |                       |  |
| <i>Brassica rapa</i> ssp. <i>oleifera</i><br>EC242660-61, EC242646,<br>EC253287, EC253291   | Turnip rape,<br>Torja | AICORPO, 1989  |
| <i>B. rapa</i> var. yellow sarson<br>PYS6, BINA 1,2   |                       | Kolte, 1987;<br>Rahman <i>et al.</i> , 1987  |
| <i>B. rapa</i> <i>rapifera</i><br>Edmonton ACC  | Turnip                | Conn <i>et al.</i> , 1988  |
| <i>B. carinata</i><br>PPSC1, HC1, EC25381,<br>EC253282, EC253284, EC253826,<br>S67, PC3, PC5, CE9   | Ethiopian mustard     | AICORPO 1989;<br>Bhowmik and Munde,<br>1987; Kidane and<br>Bekele, 1986  |
| <i>B. napus</i> spp. <i>oleifera</i><br>Altex, Gulivar 1, Karat, Narde,<br>Midas, Primer, Tower, WRG15,<br>Westar, Wei Bull - 541, 521,<br>Vuindsok, Regent 1, GS-1-1,<br>GS7027, GSL-1501, 1506, 1508,<br>1513, HNS1, HNS3, Vestal | Oilseed rape          | AICORPO, 1989; Conn<br><i>et al.</i> , 1987; Conn and<br>Tewari, 1989a; Kumar<br>and Kumar, 1989;<br>Rozej, 1974<br>Romero-Munoz<br>and Jimenez, 1979;<br>Saharan 1992a,b;<br>Stankova, 1972 |
| <i>B. juncea</i><br>BEC107-109, 112, EC129126-1,<br>PR8805, PHR 1, KRV Tall, RC781,   | Indian mustard        | AICORPO, 1989; Kolte,<br>1985; Saharan,<br>1992a,b   |
| <i>B. oleracea</i> var. <i>alboglabra</i>   | Chinese kale          | Munde and Bhowmik,<br>1985; Zaman and<br>Biswas, 1987  |
| <i>B. oleracea</i> var. <i>botrytis</i><br>Lines 1-6-1-2, 1-6-1-4,<br>Pusa Shubhra  | Cauliflower           | Singh <i>et al.</i> , 1987   |
| <i>B. oleracea</i> ssp. <i>gemmifera</i><br>Cambridge No. 5   | Brussels sprouts      | Berry and Lennard,<br>1988; Williams and<br>Pink, 1987   |

|   |  |  |
|---|--|--|
| <i>B. alba</i>  | White mustard                                  | Kolte, 1987; Saharan, 1992a,b  |
| <i>B. hirta</i>   |  | Brun <i>et al.</i> , 1987  |
| <i>Camelina sativa</i><br><i>Capsella bursa-pastoris</i><br><i>Neslia paniculata</i>                | False flax<br>Shepherd's purse<br>Ball mustard | Tewari and Conn, 1993;<br>Conn <i>et al.</i> , 1991;<br>Jejelowa <i>et al.</i> , 1991;<br>Grontoft, 1986 |
| <i>Sinapis alba</i><br>S10001, S1004-10   | White mustard                                  | Kolte, 1985b;<br>Rai <i>et al.</i> , 1977  |
| <b><i>A. brassicicola</i></b>   |  |  |
| <i>B. oleracea</i> var. <i>botrytis</i><br>PI'S 231208-209, 217934,<br>231209, 267725, Pusa Shubhra | Cauliflower                                    | Braverman, 1971;<br>Singh <i>et al.</i> , 1987   |
| <i>B. oleracea</i> var. <i>gemmifera</i>  | Brussels sprouts                               | Braverman, 1976, 1977  |

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1987a,b; Conn *et al.*, 1991; Grontoft, 1986; Jejelowa *et al.*, 1991; Saharan, 1992b; Tewari and Conn, 1993). Sources of resistance to *A. brassicae* and *A. brassicicola* in different host species from different areas of the world are given in Table 15. In India, *Brassica* genotypes viz., CSR43, CSR142, CSR142-2, CSR343, CSR448, CSR622, CSR741, Gulivar, KRV-Tall, Midas, PHR1, RC781, TMV2, Tower and YRT3 were found consistently to have field resistance after testing for many years at different locations under uniform disease nursery trials (Kolte, 1985b, 1987; Saharan, 1984, 1992a,b; Saharan and Chand, 1988).

#### **g. Development of Resistant Cultivars**

Attempts to obtain resistance in agronomically superior genotypes of *B. juncea* through mutagenic agents such as gamma rays have met with little success. Verma and Rai (1980), exposed the seeds of *B. juncea* cultivars Varuna, PR5 and RS3 to gamma-rays and obtained a mutant with 60 KR dose which is promising in yield and resistant to *A. brassicae*. Rahman *et al.* (1987) selected mutant BINA 1 resistant to *A. brassicae* from *B. rapa* cultivar YS52 treated with EMS in Bangladesh. Das and Rahman (1989) obtained the highest frequency of variation for alternaria blight in M2 by irradiating seeds of yellow sarson cultivar YS52 with 70 KR gamma rays. Sharma (1990) identified mutants in M2 population of cultivars Varuna and Kranti as resistant to alternaria blight on the basis of small lesion size under natural epiphytotic conditions. MacDonald and Ingram (1985) selected and regenerated plants from calli resistant to partially purified culture filtrates of *A. brassicicola*. According to Katiyar and Chopra (1990), a somaclonal variant obtained from an exotic yellow-seeded accession of *B. juncea* retained resistance to disease

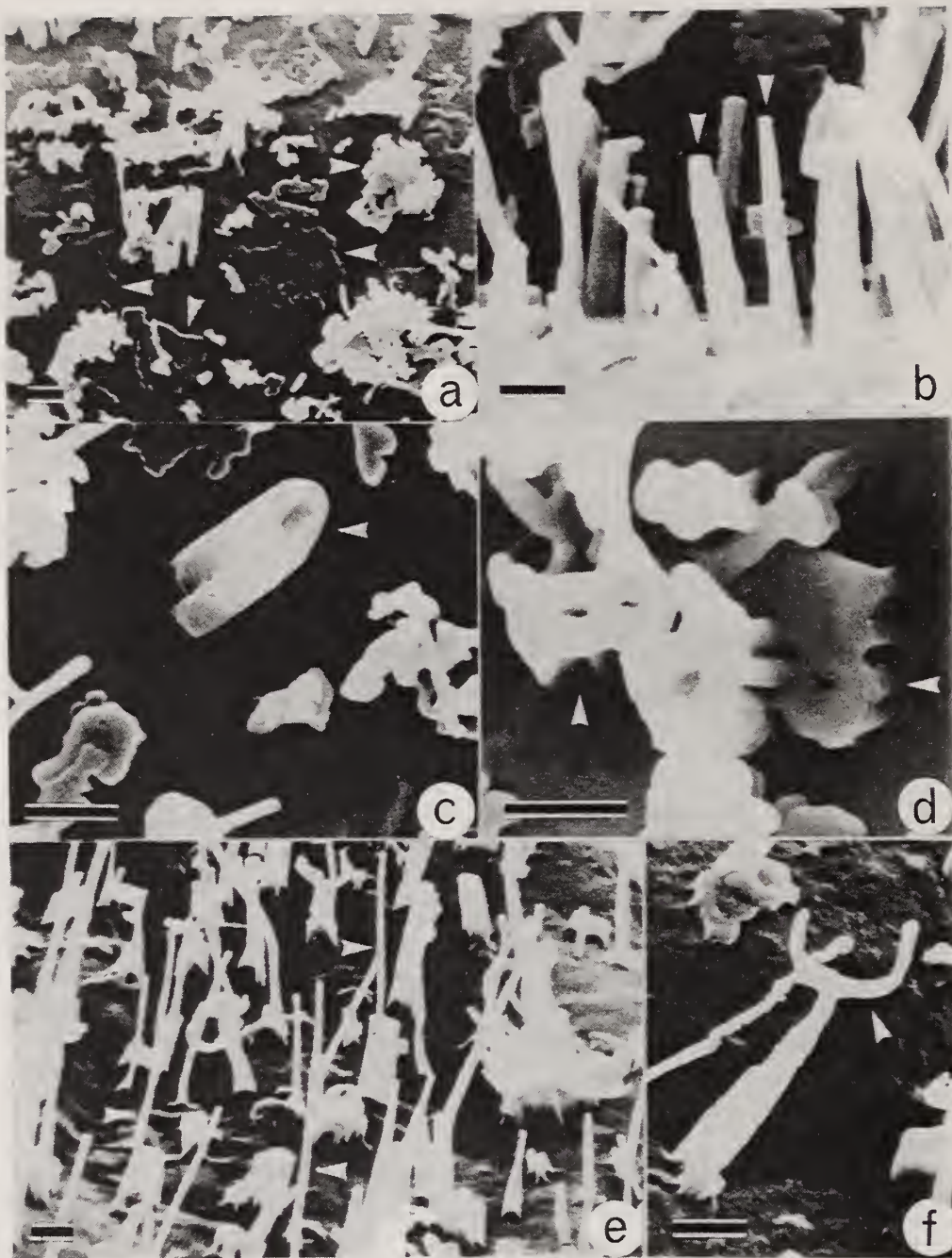


Fig. 21. (a) Adaxial surface of an upper leaf of *Brassica napus* cv. Altex showing plate-like wax crystals (arrows); (b) stem surface of cv. Altex from the middle of a plant showing rods (arrows); (c) abaxial surface of an upper leaf of *B. rapa* cv. Tobin showing a fused rod (arrow); (d) adaxial surface of an upper leaf of *B. napus* cv. Westar showing fused rods (arrows) and growth rings in wax crystals; (e) adaxial surface of an upper leaf of cv. Tobin showing filamentous wax crystals (arrows); and (f) adaxial surface of a middle leaf of cv. Tobin showing a branched filamentous wax crystal (arrow). The plant surfaces depicted in a-f were prepared for SEM by the air-drying method. Bar = 1  $\mu$ m (Courtesy: Conn and Tewari, 1989).



similar to the parental material. The transfer of resistance from different sources in different *Brassica* crops is possible and is being done through the following conventional and modern techniques all over the world.

1. Germplasm evaluation for sources of resistance at National and International levels.
2. Selection for disease resistance through a) pure line selection, b) mass selection, c) modified recurrent mass selection, and d) recurrent selection.
3. Breeding for disease resistance by increasing the level of resistance through a) multiple crosses, b) recurrent selection, c) diallele crossing, and d) selective mating system.
4. Transfer of resistance by a) intraspecific pedigree, backcross and modified recurrent mass selection methods, and b) interspecific genome substitution, chromosome substitution and gene introgression.
5. Transfer of resistance through mutation breeding.
6. Use of biotechnological and genetic engineering techniques such as a) genome manipulation, b) manipulation of cytoplasmic genomes, c) use of transformation and foreign gene expression techniques, and d) embryo rescue techniques for wide hybridization.

## 11. PHYTOTOXINS

*Alternaria brassicae* has a multitoxin system and produces at least three phytotoxins. One phytotoxin is the cyclodepsipeptide, destruxin B having molecular formula  $C_{30}H_{51}N_5O_7$  and a molecular weight of 593 (Ayer *et al.*, 1987; Ayer and Pena-Rodriguez, 1987a,b; Bains, 1989; Bains and Tewari, 1986, 1987, 1989). The effects of destruxin B are similar to *A. brassicae* on various brassicas (Figs. 22, 23; Tewari, 1991). Destruxin B is also produced by *Metarhizium anisopliae* and some other fungi and has insecticidal properties as well (Gupta *et al.*, 1989). Destruxin B is a tripeptide based on isoleucine, proline and  $\beta$ -alanine.

The second phytotoxin, homodestruxin B with molecular formula  $C_{31}H_{53}N_5O_7$  (Ayer and Pena-Rodriguez, 1987a,b) affects both hosts and non-hosts of *A. brassicae* (Bains, 1989).

Recently, reversed-phase chromatography on Sep-Pak C18 cartridges followed by high-performance liquid chromatography (HPLC) has successfully been used for the separation and detection of destruxins from *M. anisopliae* (Samuels *et al.*, 1988). Using a modification of this method, three destruxins viz., destruxin B, destruxin B<sub>2</sub> and homodestruxin B have been isolated from *A. brassicae* (Buchwaldt and Jensen, 1991; Buchwaldt *et al.*, 1991). According to Buchwaldt and Green (1992), destruxin B, the major phytotoxin produced by *A. brassicae*, is not host-specific as reported earlier (Bains and Tewari, 1987). This toxin causes necrosis and chlorosis on 30 species of host and non-host plants. Two minor destruxins, homodestruxin B and destruxin B<sub>2</sub> are phytotoxic to leaves of *B. napus*. However, there are significant differences between taxonomic plant groups in their sensitivity to destruxin B. *Brassica* species are most sensitive to the toxin, and sen-



Fig. 22. Symptoms caused by inoculation of *Brassica napus* cv. Altex leaf with *Alternaria brassicae* (left leaf). The right leaf is the control (Courtesy: Tewari, 1991).



Fig. 23. Symptoms caused by application of destruxin B on *Brassica napus* cv. Altex leaf (left leaf). Compare with figure 22. The right leaf is the control (Courtesy: Tewari, 1991).

sitivity decreases as relatedness of plant groups become more distant. The dilution-end-point of destruxin B is 0.2–3.8  $\mu\text{g/ml}$  for the most sensitive host species, and 15–120  $\mu\text{g/ml}$  for the least sensitive. The sensitivity of non-host species is between 15 and 750  $\mu\text{g/ml}$ . Destruxin B shows a high degree of biological activity (Bains, 1989; Bains and Tewari, 1989). In detached rapeseed leaf bioassay, the limit of detection of destruxin B lies between 15 and 30  $\mu\text{g/ml}$ , and it is possible to distinguish pathogen susceptibility differences among host species (Bains and

Tewari, 1987). The symptoms caused by destruxin B appear to be light-dependent. Destruxin B appears to be a virulence factor, contributing to the aggressiveness of *A. brassicae* by conditioning the host tissue and thereby determining the susceptibility of the host. Homodestruxin B is able to affect both the host and nonhosts of *A. brassicae* and therefore functions as a non-host specific toxin. Homodestruxin B, differs from destruxin B by the replacement of a methyl with an ethyl group. This structure-function relationship is of great interest and needs further investigation (Fig. 24).

Somaclonal variation produced in cultured cells and tissues provides a good system for selection of new disease-resistant genotypes of brassicas using destruxin B. As a host specific toxin, destruxin B is the primary determinant of the blackspot disease of the Brassicaceae. It should be possible to use destruxin B as a selection agent for disease resistance in tissue culture systems. Tissue culture host systems with cell walls would be suitable for this purpose because the protoplasts are insensitive to the toxin (Bains and Tewari, 1989).

In the presence of destruxin B, leaf discs of the susceptible host *B. rapa* var. yellow sarson, lose significantly more electrolytes than the moderately resistant *B. rapa* var. *rapifera* (turnip). The effects takes up to 48 h to become apparent, and the lowest concentration of toxin detectable by this bioassay method is 10 µg/ml. The delayed electrolyte leakage indicates that perhaps the target site of the toxin in the

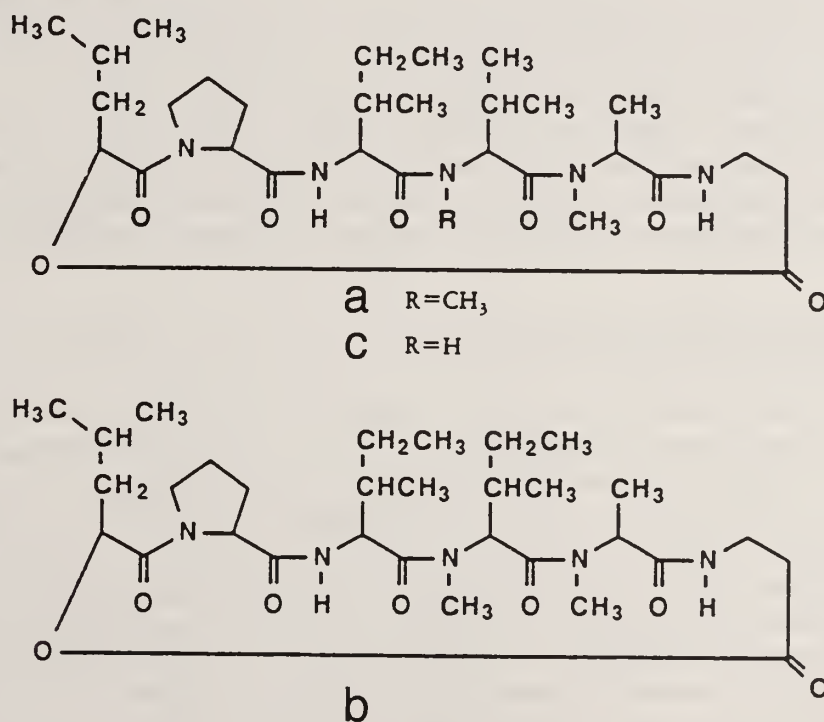


Fig. 24. Structural formulas of three destruxins produced by *Alternaria brassicae* (a) Destruxin B; (b) Desmethyldestruxin B; and (c) Homodestruxin (Courtesy: Ayers, Bains, Pena-Rodriguez and Tewari, 1987).



host cell is not situated directly on the cell membrane, because of the delay for the permeability change to occur and the electrolyte leakage to start. The mesophyll protoplasts of both susceptible (*B. rapa* var. yellow sarson) and resistant (*C. sativa*) hosts are not affected by destruxin B, even at a concentration of 100 µg/ml. This indicates that the target site of the toxin must be in the cell wall or the periplasmic space. Germination and tube growth of pollen grains of the susceptible host (*B. rapa* var. yellow sarson) has been found to be very sensitive to destruxin B concentration as low as 2.5 µg/ml for 30 minutes. Complete inhibition of these processes occurs at a concentration of 10.0 µg/ml (Bains and Tewari, 1989). These processes are not completely inhibited in the resistant host (*C. sativa*), even at a toxin concentration of 100 µg/ml.

## 12. TECHNIQUES

### a. Stem Explant Culture Inoculation Techniques

The technique consists of cutting 6–15 cm terminal shoots bearing leaves and/or flowers and pods at 3 to 5.3 growth stage of the plants. The cut end of such shoots are immediately immersed in tap water in Erlenmeyer flasks or test tubes. Care is taken to see that cut ends of the shoots remain suspended in water. The leaves and/or pods are spray-inoculated with *A. brassicae* conidial suspension. Inoculated shoots are incubated in a polyethylene moist chamber (90–95% RH) at 20–30°C maximum and 6–14°C minimum temperatures in diffused light for 72 h. Special care is taken to make up the loss of water in flasks or test tubes used and also by slowing down the transpiration rate through shaded moist conditions. Symptoms of the disease appear within 4–5 days of inoculation. In 15–20 days the cut ends of test shoots initiate root formation with profuse rooting in 30 days. New shoots may generate from the axillary buds. Such stem explants can be transferred to soil in pots where pod formation with good seed development can be possible. This technique is advantageous over others in its simplicity and obtaining the seeds of test genotypes in the same season for further use (Kolte and Yadav, 1990).

### b. Leaf Disc Inoculation Technique

Test plants are grown under controlled conditions. From the excised healthy leaves of such plants, leaf discs 2.5–3.0 cm diameter are cut and are washed thoroughly by rinsing 5–6 times with distilled water in test tube of wider diameter (>3 cm). To insure elimination of surface contaminants, fresh aliquots of water are used for each rinse. Surface sterilized discs are placed on a 4 mm thick layer of 1% plain sterilized agar petri dishes containing 60 ppm benzimidazole. The adaxial surface is placed against the agar and the abaxial surface is drop-inoculated with 0.01–0.02 ml of conidial suspension (Humperson-Jones and Phelps, 1989). Suitable controls with distilled water drop-inoculated discs are maintained. Excess moisture from the leaf discs is removed with absorbant paper. The inoculated discs are incubated under continuous cool white fluorescent light ( $5.5 \mu\text{EM}^2 \text{ S}^{-1}$ ) at 18–24°C temperature for *A. brassicae*, and at 20–30°C for *A. brassicicola* for 4–5 days. The symptoms in the form of lesions are observed to record the reaction of test genotypes.

### c. Detached Leaf and Pod Inoculation Technique

In the test plants third or fourth leaves (at 3.3 to 4.2 growth stages) and pods (at 5.3 to 6.5 growth stages) are detached for inoculation. An approximately 3 mm<sup>2</sup> of the upper surface is gently scratched with the tip of a pasteur pipette, and 50 µl drop of spore suspension ( $5 \times 10^4$  spores ml<sup>-1</sup>) placed on the scratched area (Bains and Tewari, 1987). Five leaves/pods from each test genotypes are inoculated at four points at random. Suitable checks with sterile water droplet inoculation are kept. The inoculated leaves/pods are incubated for 72h on a moist filter paper in petri dishes at 25–26°C temperature under continuous cool white flurescent light ( $5.5 \mu\text{EM}^2 \text{ S}^{-1}$ ). The symptoms in the form of lesions develop within 4–5 days after inoculation.

### d. Detached Leaf Inoculation Technique

In a laboratory study Bansal *et al.* (1990) screened *Brassica* species against *A. brassicae* on the basis of the size of lesions caused on detached wounded leaves. The leaves are wounded to eliminate the protective effect of the wax layer in order to examine the reaction of the underlying tissues. Ten plants from each genotype are grown individually in 5 x 5 cm pots containing soil-free growth medium in the greenhouse (ca. 20/16°C day/night temperature, 18h photoperiod achieved with supplementary illumination provided by 400W high pressure sodium lamps).

The isolate of *A. brassicae* is cultured on V8-agar supplemented with Rose Bengal (0.4mg L<sup>-1</sup>) and incubated at room temperature under an 18 h photoperiod. For plant inoculation, aqueous spore suspensions ( $4\text{--}5 \times 10^5$  spores ml<sup>-1</sup>) are prepared from 10- to 14-day-old culture. The fully expanded fourth leaf is removed from each plant (ca. 18 d after sowing) and placed in a clear plastic container lined with water-soaked paper towels. The leaves are punctured on both sides of the midrib with a number 24-needle inoculator which induces circular wounds of ca. 3 mm in diameter and a 25 µl drop of spore suspension is applied to each wound (50 µl per leaf) with an Eppendorf micropipette. Control inoculations are performed on other leaves with sterile distilled water. The plastic containers are sealed with clear plastic wrap and incubation is performed at room temperature under continuous light. Four days after inoculation, lesion diameter, including the chlorotic zone, when present, is recorded.

### e. Greenhouse Method for Testing Resistance

Small differences in attack of *A. brassicae* on *Brassica* species are difficult to detect in the field. Therefore a more detailed method under greenhouse conditions was developed by Grontoft and O'Conner (1990). Plants of test genotypes are grown in pots. When the first true leaf emerges the plants are thinned to one healthy plant per pot. From the time of sowing to the time of evaluations of genotypes, the temperature is kept constant at approximately 20°C. The plants are inoculated at the three-leaf stage using *Alternaria* inoculum grown on PDA for 3 weeks at 18°C. Inoculation is performed using small agar discs (10 mm) cut from just behind the mycelium growth front with a corkborer. The discs are placed with



the fungus growth sides against the upper side of the 2nd leaf over a 4 mm hole. A second hole of similar size is cut on the other side of the midvein. This hole is used to control if the size of necrotic lesions are in any way influenced by leaf surface area growth. Immediately after inoculation each plant is covered with plastic in order to increase relative humidity and enhance fungal growth. The genotypes are evaluated 7 days later by measuring the diameter of the necrotic lesions on the leaves.

#### **f. Brassica Germplasm Screening Technique for Resistance Through AB-Toxin**

In this technique sensitivity of test genotypes to the alternaria blight toxin is correlated with the disease reaction. It is believed that level of sensitivity of different *Brassica* species to the toxin is similar to the order of susceptibility of these to *A. brassicae* (Fig. 22; Bains and Tewari, 1987). It can cause symptoms of varying severity, dependent upon host genotype, which range from severe chlorosis and necrosis to almost no visible chlorosis (Tewari and Bains, 1988). A purified preparation of AB-toxin is obtained from the single-spore culture of *A. brassicae* (Bains and Tewari, 1987). Test genotypes are grown under controlled conditions. On the detached third or fourth leaf (3.3 to 4.2 growth stage) 3 mm<sup>2</sup> area is gently scratched with a pasteur pipette, and 20 µl of the purified toxin is applied. The leaves are incubated on a moist filter paper in a petri dish on laboratory benches under continuous fluorescent light. Within 48 h of incubation reaction of the test genotypes is expressed.

*In vitro* screening of protoplasts of different *Brassica* genotypes, or screening of secondary embryoids, originally derived from a single anther embryoid through AB-toxin, have been employed since plant protoplasts are known to be more sensitive to host-specific toxins (MacDonald and Ingram, 1985, 1986). Secondary embryogenic culture lines are maintained on MS medium (Murashige and Skoog, 1962) containing 0.8% Difco agar and 2% sucrose, but without plant growth substances. When regenerants are required, secondary embryoids are transferred for 3–7 days to MS medium supplemented with kinetin and then transferred to fresh medium without growth substances for root development. Plantlets are potted in peat balls and placed in high humidity in the greenhouse until they are established. Leaves from such plants are surface-sterilized and placed in plastic boxes on moist filter paper. Two drops (10 µl each of suspension of approximately  $5 \times 10^5$  spores ml<sup>-1</sup> of *A. brassicicola*) are placed on each detached leaf and incubated in low continuous light (approx. 1.7 mW cm<sup>-2</sup>) at 25°C. Secondary embryoids have exhibited a wide range of reactions, including a number of resistant plants (MacDonald and Ingram, 1985, 1986; MacDonald *et al.*, 1986).

*In vitro* pollen selection (microspore culture) in brassicas for resistance to toxins from *A. brassicae* and *A. brassicicola* has been applied (Hodgkin, 1990; Tewari and Bains, 1988). The AB-toxin obtained from *A. brassicae* at 12.5 µg/ml completely inhibits the germination of pollens of highly susceptible yellow sarson (*B. rapa*), whereas pollen of highly resistant *C. sativa* germinates even at 100 µg/ml of the toxin (Tewari and Bains, 1988). Recently, Hodgkin (1990) described *in vitro* pollen selection in *B. napus* for resistance to phytotoxic compounds from *A.*



*brassicicola*. Pollen samples from *B. napus* cultivars "Arran" and "Herkules" are incubated for 1 h in a germination medium or in a medium to which 20 mg ml<sup>-1</sup> of a toxic extract from *A. brassicicola* has been added. The pollen samples are then used to pollinate cultivar Primar. A number of plants, obtained from pollinations using pollen incubated in the toxic extract, produce pollen with a significantly increased ability to germinate in medium containing 10 mg ml<sup>-1</sup> of the extract. Evidence that some selection for resistance to the toxic compounds produced by *A. brassicicola* has been obtained (Hogdkin and MacDonald, 1986).

#### **g. Semi-Selective Medium for Detecting Seed-Borne *A. brassicicola***

A semiselective medium containing benomyl, sodium propionate, streptomycin sulfate, and chloramphenicol in peptone-dextrose agar has been developed to detect seed-borne *A. brassicicola* (Wu and Lu, 1984a). This medium is more sensitive than the blotter method to detect *A. brassicicola* in cabbage seeds. Seeds incubated on this medium at 22°C for 7 days produce colonies of *A. brassicicola* which appear velvety olivaceous brown with abundant sporulation. There is a positive correlation between the amount of seed-borne *A. brassicicola* detected on this medium and the percentage of diseased seedlings in autoclaved field soil or in peat.

#### **h. Radish Root Extract Agar for *A. brassicae* Sporulation**

The medium can be prepared by cooking grated radish roots (200 g) in distilled water (500 ml) and adding agar solution (20 g in 500 ml) to the radish root extract filtrate. In order to get the spores of the fungus in culture, the radish root extract may be supplemented with sucrose at 1% or with mannitol at 1%. Significantly larger fungal colonies with profuse sporulation are obtained on this medium (Thakur and Kolte, 1985).

#### **i. Technique for Inducing Sporulation of *A. brassicae***

Stock cultures of *A. brassicae* derived from single conidium are stored at room temperature on oxoid potato-dextrose agar (PDA) slopes under sterile paraffin oil. Petri dish cultures of individual isolates are grown for 7 days at 25°C on PDA. From these, a 2 cm<sup>2</sup> mycelium piece is removed, placed in a sterile 25 ml masticator with 10 ml of sterile distilled water, and macerated at maximum speed for 1 minute. Portions (0.2 ml) of the resulting macerate are transferred to 9 cm diameter triple vented polystyrene petri dishes containing 10 ml of diluted autoclaved V-8 juice. Three replicates of each of 8 field isolates are incubated separately under near ultraviolet (UV) radiation, white light and in darkness, at 25°C for 7 days. The sources of near UV radiation and white light are two 40W Philips radiation BLB (310–420 nm) and two 85 W Sylvania cool white (310, 330, 350, 750 nm) fluorescent lamps, respectively. After 2–3 d incubation, the mycelium in the petri dish forms a loosely interwoven mat of hyphae throughout the liquid medium, producing a solid gelatinous matrix. As the cultures then slowly dehydrate, the pinkish-white mycelia turn dark olivaceous brown, which can be correlated with the production of melanized conidiophores and conidia. After 7 days of incubation the cultures are dehydrated to 90% of their original mass (Senior *et al.*, 1987).

Conidia are removed by flooding individual plates with 10 ml of a 0.01% (V/V) sterile Tween 20 solution and rubbing the mycelial mat with a sterile glass spreader for 1 min. When the loosely interwoven mycelial mat is evenly suspended, the contents of the petri dish are transferred to sterile 30 ml universal bottles and mixed using a whirlimixer for a further 3 min. The resulting suspension is filtered through 2 layers of sterile lens tissue (Whatman No 105) and washed with a further 5 ml of 0.01% sterile Tween 20 solution. The conidial suspension is then centrifuged for 10 min at 2750 g (rav. 7.5 cm) after which the supernatant is discarded and conidia are resuspended in 5 ml of 0.01 percent sterile Tween 20 solution and counted with a haemocytometer. The mean of 6 counts is taken for each replicate. Conidial germination is assessed by preparing spread plates (0.1 ml aliquots of conidial suspension spread over PDA plates) and incubating them for 24 h at 20°C. For each isolate, 200 conidia are examined and the percentage germination is calculated (Senior *et al.*, 1987).

**j. Brassica Callus Culture to Induce Sporulation in *A. brassicae***

Twenty-five surface-sterilized *B. juncea* seeds are aseptically transferred to a 250 ml conical flask containing 30 ml sterile Murashige-Skoog (MS) medium (Murashige and Skoog, 1962). The seeds are incubated in the dark for 3 days and then in the light (300 lux) for 2–3 days until seedlings reach to the neck of the flask. Cotyledons are then excised, cut into two pieces each and transferred to a 100 ml conical flask containing 30 ml modified MS replacing IAA and kinetin with 2 mg/L NAA, 3 mg/L 2,4-D and 0.2 mg/L of 2- iPr (2 - isopentenyl- purine riboside). The inoculated flasks are incubated at 25°C (±2) under continuous light (300 lux). Callus cultures are subcultured every 3 weeks. A nonsporulating isolate of *A. brassicae* is grown in culture tubes in radish-mannitol agar (RMA) medium. RMA medium is prepared in the same way as PDA medium except that potato and dextrose are replaced by radish and mannitol, respectively. The fungus is subcultured every 15 days. Two-week-old calluses are transferred onto agar (10 g/L), modified MS medium or modified MS medium supplemented (after sterilization) with 100 mg/L Captafol in 9 cm diam petri dishes (one callus per dish) and maintained at 25°C (±2) under continuous light (300 lux). After 2 days of incubation, a small disc (approx. 1 mm diam) of fungal mycelium is cut from the growing edge of the fungus culture and placed at the top of each callus (Joshi *et al.*, 1988).

**k. Method of Estimating *A. brassicicola* in Seed**

Naturally infected cabbage seeds are incubated for four days at 18–21°C with alternating light and dark periods, on filter papers moistened with 2000 ppm aqueous solution of Dow sodium salt of 2,4-dichlorophenoxyacetate (2,4-D). When seeds are placed closer than 6 mm apart, or incubated longer than four days, exaggerated pathogen counts occur. Two thousand ppm 2,4-D efficiently inhibits seed germination and does not reduce pathogen count. Optimum temperature range is 20–25°C; above this range contaminant fungi are a problem. Continuous light or alternate light and dark is preferable to continuous dark. Excessive moisture is detrimental. Pretreating seeds with ethanol (EtOH) or sodium hypochlorite (NaOCl) effectively reduces rapid growing contaminant fungi but significantly reduces pathogen count (Bassey and Gabrielson, 1983a,b). However, other common methods



in use for detection of seed-borne inoculum are a) deep-freezing blotter method, b) germination test, c) blotter method, and d) agar plate method (Vannacci, 1981).

### **1. Identification of Fungicide Antagonists in Leaf Exudate**

Attempts have been made to identify the fungicide antagonists in exudate extracts of leaves of wallflower (*Cheiranthus cheiri*) (Beynon and Brown, 1969). In extracts prepared by immersing wallflower leaves in water, the antagonism of the activity of the fungicide ethylene thiuram disulphide (ETD) against the spores of *A. brassicicola* has been shown to be due to the presence of glucose and fructose. These compounds are present in the extracts at concentrations corresponding to 0.015 µg of glucose and 0.002–0.005 µg of fructose for every square centimeter of leaf extracted. Dunn *et al.* (1969) showed that water-soluble leaf exudates extracted from apple, vine, tomato, potato and wallflower reduced the fungitoxicity of ETD to *A. brassicicola*. The spectrum of the leaf exudate effect is wide and the magnitude of the effect can vary in each host-parasite-fungicide relationship. It seems that a large proportion of fungicide treatments may be reduced in their efficacy by antagonistic materials which either occur on the plant surface or are readily and quickly available from intact plant tissue. It is likely that the active principle affects the fungal spore, either by reducing its permeability to fungicides or by influencing some part of the metabolism involved in spore germination. Stimulation of the spore metabolism could activate systems capable of detoxifying alien chemicals. The major part of the exudate effect observed is due to glucose.

### **m. Ovary and Ovule Culture**

Four to 12-day-old ovaries are excised and sterilized with 0.25% aqueous Hg Cl<sub>2</sub> for 8–10 min followed by three washings with sterilized distilled water. The base of the pedicel is cut with a blade and ovaries are cultured on MS medium (Murashige and Skoog, 1962) supplemented with various concentrations and combinations of growth regulators. Three to five ovaries are cultured in each tube containing 15 ml of medium solidified with 0.8% agar and incubated at 25°C (± 1) in an illuminated room (700 lux). Thirty-five days after culture, the ovaries are cut open to collect hybrid seeds. The F<sub>1</sub> seeds from the cultured ovaries are germinated in petri dishes and the seedlings are transferred to pots in glass house (Sharma and Singh, 1992).

For ovule culture, 12–17 days after pollination, the ovaries are surface sterilized and cut open aseptically under dissecting microscope. Ten to twelve excised ovules are cultured in test tubes containing MS and modified PC-L2 medium (Philips and Collins, 1979). Observations on seed formation are recorded 15 days after culture. The seeds are first germinated in petri dishes and transferred to 8 in diameter pots in the glass house. The F<sub>1</sub> hybrids are evaluated for their reaction to *A. brassicae* by inoculating with spore suspension (2000–2500 spores ml<sup>-1</sup>) in the glass house (Sharma and Singh, 1992).



### 13. DISEASE MANAGEMENT

No single method or approach in current use is feasible, viable, stable, effective and economical in dealing with any host-pathogen system. Therefore, it is necessary to integrate all the methods of plant disease control which are available, including; cultural, chemical, biological and host resistance to effectively manage alternaria diseases of brassicaceous plants (Eastburn, 1989; Saharan, 1992a).

#### a. Cultural Measures

Among the common cultural practices, use of clean, large, healthy seed, seed treatment, long crop rotation, sanitation, weed control, planting at recommended times, use of balanced nutrients, proper plant density, proper drainage in the fields, use of tolerant/resistant cultivars and application of chemicals at proper time with adequate foliage coverage have been advocated to control alternaria diseases of brassicaceous plants (Channon and Maude, 1971; Chupp, 1925; Chupp and Sherf, 1960; Daebeler *et al.*, 1981, 1987; Dixon, 1981; Kolte, 1985b; Lapis and Ricaforte, 1974; Saharan and Chand, 1988; Singh, 1987; Thomas, 1984). Seed infection can largely be controlled by hot water treatment at 50°C for 18 min (Schimmer, 1953) or by soaking *Brassica* seeds in a 0.2 percent aqueous suspension of Thiram for 24 h at 30°C (Maude, 1978; Maude and Shuring, 1968; Maude *et al.*, 1969). In tropical countries such as India, aging of *Brassica* seeds for 5–6 months at temperatures above 35°C eliminates seed infection and seed-borne inoculum (Chahal, 1981; Kolte, 1985b). Alternaria pod blight of radish is significantly less in the early transplanting of 15th November under Indian conditions (Sandhu *et al.*, 1985). In Sweden *A. brassicae* occurs more frequently in areas with both summer and winter oilseed crops than in areas with mainly summer crops (Morner, 1980). In India early planting of toria (*B. rapa*) in September usually escapes *Alternaria* infection. In the Lujaska area of Leningrad (USSR), there is a lower incidence of *A. brassicae* on cabbage if it is transplanted between 20–30 June. Application of fertilizers containing humus, 45% peat and 5% mullein, plus 3.9 g ammonium nitrate, 3.4 g potassium chloride and 8.1 g superphosphate/100 added to 110 g organic matter reduces the disease better than the organic manure alone (Kupryanova, 1957).

To avoid storage and transit losses in cabbage and cauliflower, heads should be handled carefully to avoid bruising and surface moisture allowed to evaporate before storage. The storage house should be kept at 33–34°F and ample ventilation provided to reduce humidity (Walker, 1927).

#### b. Seed Treatment

i) **Hot-water treatment:** Excellent control of *A. brassicae* in white cabbage and cauliflower seeds has been obtained in Danish experiments from 1934–1936 by 30 minutes immersion in water heated to 45°C or for 20 minutes at 50°C, or even 30 minutes at 40°C. This treatment improves germination by up to 13% along with elimination of other fungi such as *Penicillium* and *Mucor* spp. (Nielsen, 1936). Hot water treatment of seeds at 50°C for 30 minutes controls the disease in cabbage (Walker, 1952). According to Ellis (1968a,b), hot water treatment for 25 minutes at

50°C eliminates *Alternaria* infection from brassicaceous seeds. Seed treatment at 50°C for 20 min is highly effective in controlling the seed-borne fungi including *A. brassicae* of raya (*B. juncea*) without any pronounced effect on the germination of the seeds (Randhawa and Aulakh, 1984).

**ii) Chemical treatment:** For controlling seed-borne *A. brassicae*, *A. brassicicola*, *A. raphani* and *A. alternata* infections in brassicaceous plants, seed dressings with Benlate, Dithane M-45, Dithane Z-78, Difolatan, Dichlofluanid, Delan C, Bristan, Bavistin, Brassicol, Captan, Fenpropimorph, Granosan M, Quinolate, Merpan, Rovral, Thiram, Vitavax, Thiride and Zineb, have been found to be very effective (Atkinson, 1950; Chahal *et al.*, 1977; Champion *et al.*, 1979; Chirco and Harman, 1979; Ellis, 1968a,b; Gupta and Saxena, 1984; Holtzhausen, 1978; Humpherson-Jones *et al.*, 1980, 1981, 1983, 1984; Kanwar and Khanna, 1979; Kumar and Singh, 1986; Maude and Humpherson-Jones, 1980a,b, 1984; Maude *et al.*, 1972, 1984; Mridha and Safa, 1985; Randhawa and Aulakh, 1982; Wu and Lu, 1984b).

Soaking *Brassica* seeds in an 0.2% aqueous suspension of Thiram for 24 h at 30°C eradicates *Alternaria* infection (Maude, 1978; Maude and Shuring, 1968; Maude *et al.*, 1969). In New South Wales two samples of infected seed were treated with iprodione at 1.25 g a.i. Kg<sup>-1</sup> seed and the level of seed-borne *A. brassicae* was reduced from 21 to 2% and from 14 to 4.5%. The same treatment completely eliminates *A. brassicae* from pieces of trash mixed with seed (Stovold *et al.*, 1987). Seed treatment with iprodione, which virtually eradicates seed-borne infection of *Alternaria* spp. in the Brassicaceae, is now used to treat basic seed stocks in England (Maude and Humpherson-Jones, 1980a,b). This practice is most effective in crops grown in isolation from maturing brassicaceous seed crops. However, where seed crops are grown successively and in close proximity, as it often occurs in the main seed producing areas, infection may arise from spores released from diseased seeding crops during cutting and threshing and disseminate by the wind to young crops destined for seed production in the following year (Humpherson-Jones and Maude, 1982a, 1983). In such situations additional control measures are necessary.

According to Maude and Suett (1986), use of a prototype fluidised-bed seed treater to apply iprodione in a polymer film coat to cabbage seeds infected with *A. brassicae* and *A. brassicicola* has been found effective. Analysis of seed-to-seed variability in dosing demonstrated the greater accuracy of fungicide application and control of *A. brassicae* by film-coating than by slurry or dust methods. Application of polymer film-coat slightly reduces superficial inoculum of *A. brassicicola*.

Seed treatment of mustard with Bavistin, Difolatan (1.5 g/Kg) and Dithane M-45 (2 g/Kg) eradicates *A. brassicae* infection. A minimum period of 24 h is necessary for obtaining effective control of the pathogen in seeds (Kumar and Singh, 1986). Seed treatment with iprodione (1.35–2.5 g a.i./Kg) and fenpropimorph (0.625–2.5 g a.i./Kg) virtually eliminated *A. brassicicola* in naturally infected *Brassica* seeds without affecting seed germination in laboratory and seedling tests (Maude, 1983, 1986).

In Finland *A. brassicicola* on chinese cabbage (*B. pekinensis*) is effectively controlled by dressing seed with Thiram or powdered *Streptomyces griseoviridis*



(Valkonen and Koponen, 1990).

According to Crisan and Pall (1986) seed treatment with Vitavax, Quinolate, Dichlofluanid, Zineb, and Merpan completely inhibits *A. alternata* infection in cauliflower seed. In India, seed-borne infections of *A. alternata* in *E. sativa* are controlled by treating the seed with Agrosan GN and Dithane M-45 at 0.3% (Gupta and Saxena, 1984).

**iii) Bioagents treatment:** Seed treatment with Mycostop, a powdery formulation of spores and mycelium of *Streptomyces griseoviridis* controls *A. brassicicola* damping-off in cauliflower and cabbage (Fig. 25; White *et al.*, 1990). Biocontrol of seed-borne *A. raphani* and *A. brassicicola* in radish has been obtained through other antagonists (Fig. 26; Vannacci and Harman, 1987).

### c. Chemical Control

Inhibition of *A. alternata*, *A. brassicae* and *A. brassicicola* growth in culture and spore germination by Bavistin, Blitane, Blitox-50, Benlate, Brestan-60, Captan, Captafol, Ceresen dry, Copper oxychloride, Carbendazim, Cupramar, Cuman L, Dithane M-45, Dithane Z-78, Difolatan, Emisan, Fytolan, Fentinhydroxide, Fostyl-al dinobuton, Griseofulvin, Rovral, Methofuroxan, Mycostatin, Polyoxin B, Polyoxin D, Thiram, Tridimefon, Wettable sulphur, Zincop, Quintozene and Ziram have been observed by several workers (Ansari *et al.*, 1990b; Chand and Jatian, 1969; Maude, 1976; Prasada *et al.*, 1970; Singh and Bhowmik, 1985; Singh and Rai, 1982; Tewari and Skoropad, 1979).

*In vitro* and *in vivo* testing of large number of chemicals (Table 16) against *Alternaria* species of Brassicaceae have been carried out (Verma and Saharan, 1993). Some of the fungicides which have been found to be effective in controlling the alternaria diseases and increasing the yield under field conditions are Baycor (0.2%), Benlate (0.1%), Blitane (0.2%), Blitox-50 (0.3%), Bordeaux mixture (0.3%), Boric acid powder (0.53%), Brestan (0.1%), Captan (0.2%), Captafol (0.3%), Cuman L (0.1%), Difolatan (0.2%), Dithane M-45 (0.2%), Dithane Z-78 (0.2%), Daconil (0.2%), Duter (0.2%), Folicur (500g a.i./ha), Rovral (0.2%), Procymidone (0.750 Kg a.i./ha), Prochloraz (500g a.i./ha) and TPTH (fentin hydroxide, triphenyl tin hydroxide (0.2%) (Ansari *et al.*, 1990b; Chahal *et al.*, 1977; Chand and Jatian, 1969; Domsch, 1957; Gaur and Ahmed, 1980; Gupta *et al.*, 1977, 1985; Howlidar *et al.*, 1985; Kaspers and Siebert, 1989; Keyworth, 1969; Kolte, 1985b; Kolte and Tewari, 1978; Lapis and Ricaforte, 1974; Maude and Dudley, 1972; Noon *et al.*, 1988; Saharan, 1991, 1992a; Shivpuri *et al.*, 1988; Singh, 1986; Singh and Bhowmik, 1985; Singh and Sobti, 1980; Verma and Saharan, 1993).



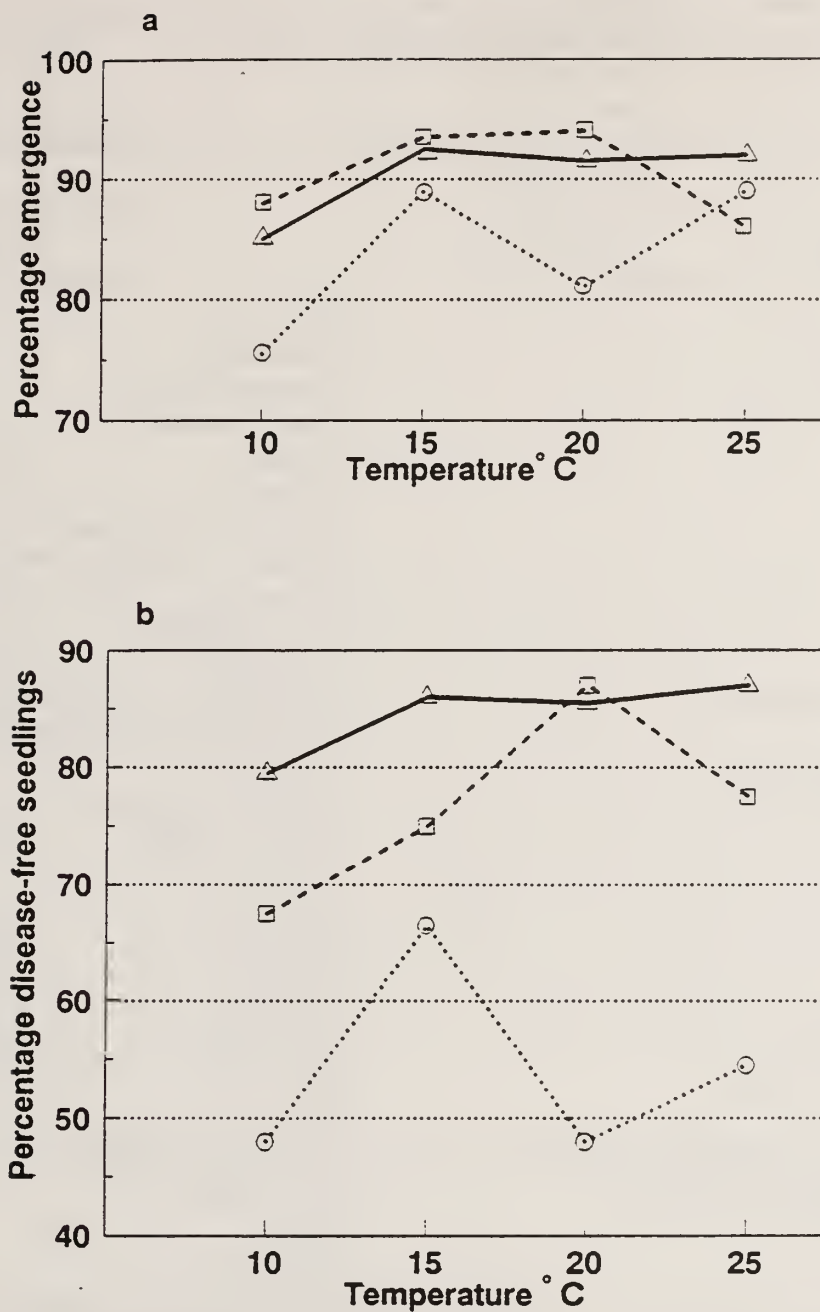


Fig. 25. The effect of *Streptomyces griseoviridis* preparation on (a) percentage emergence; and (b) percentage disease-free seedlings of cabbage cv. Celtic. 0.....0 untreated seed; □.....□ seed treated with *S. griseoviridis* preparation; and Δ.....Δ seed treated with iprodione (Courtesy: White, Linfield, Lahdenpera and Uoti, 1990).

Table 16. Chemicals tested against species of *Alternaria* attacking Brassicaceae (Verma and Saharan, 1993)

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FUNGICIDES

|                                 |                            |
|---------------------------------|----------------------------|
| Acetone                         | Euparen                    |
| Actidione                       | Fenarimol                  |
| Agrosan GN                      | Fenpropimorph              |
| Alar                            | Fentin acetate             |
| Arasan                          | Ferbam                     |
| Bafin                           | Fermate                    |
| BAS 480F                        | Folicur (Tebuconazole)     |
| Baycor                          | Folpet                     |
| Bayleton                        | Flutriafol                 |
| Bavistin                        | Formaldehyde               |
| Benlate                         | Granosan                   |
| Benz (1,2) isoxazoles           | Guazatin                   |
| Bioquin                         | Halogenated derivatives    |
| Bordeaux mixture                | Imazalil                   |
| Boric acid                      | Iprodione (Rovral)         |
| Brassicol                       | Karbam white               |
| Brestan                         | Lunasan                    |
| Bromosan                        | Malic acid                 |
| Calixin                         | Maneb                      |
| Captafol                        | Manzate                    |
| Captan                          | Merpan                     |
| Carbendazim                     | Metalaxyl                  |
| Carboxiin (Vitavax)             | Metiram                    |
| Ceresen                         | Miltox                     |
| Chlorothalonil (Daconil, Bravo) | 4-Nitrosopyrazole          |
| Copper oxychloride (Blitox)     | Nuarimole                  |
| Copper sulphate                 | Ozone                      |
| Cuman L                         | Panogen                    |
| Cupravit                        | Parzate                    |
| Cupric acetate                  | Pentachlorophenol          |
| Cuprox                          | Phygon                     |
| Cycloheximide                   | P-Methoxytetrachlorophenol |
| Delan C                         | Prochloraz                 |
| Dichlofluanid                   | Propiconazole              |
| Difolatan                       | Propineb                   |
| Dithane D-14                    | Pyrene compounds           |
| Dithane M-45 (Mancozeb)         | Quinolate                  |
| Dithane Z-78                    | Quinone                    |
| Duter                           | Ronilan                    |
| Edifenphos                      | Semesan                    |

Sisthane  
Sodium fluoride  
Spergon  
S-triazines  
Sumilex (Procymidone)  
Syllit  
Tetrahydropyrimidine  
Thiabendazole  
Thiaphanate methyl  
Thiovit  
Thiram  
Tillex  
Topsin M  
Triademefon  
Triapenthenol  
Triarimol  
Tribasic copper sulphate  
Tribasic copper zinc  
Tridemorph  
Trimiltox forte  
Vinclozolin (Ronilan)  
Wettable sulphur  
Zincop  
Zineb  
Zinc sulphate  
Ziram

#### INSECTICIDES

Cypermethrin  
Deltamethrin  
Fenvalerate  
Flucythrinate  
Metasystox  
Methyl demeton  
Permethrin  
Phosphamedon

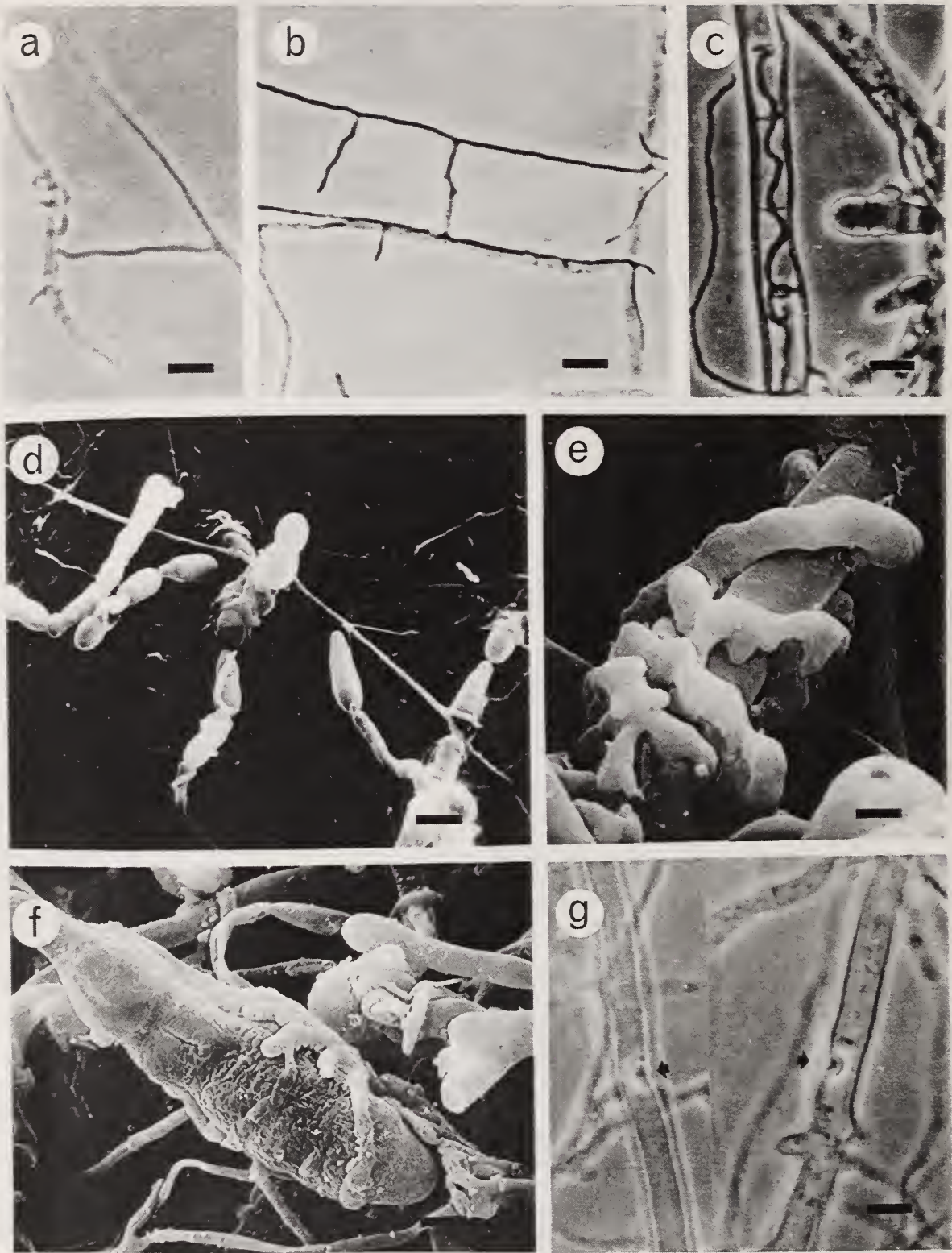
#### ANTIBIOTICS

Griseofulvin  
Mycostatin  
Mycothricin  
Polyoxin B and D

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Fig. 26. Biocontrol of seed-borne *Alternaria raphani* and *A. brassicicola*: (a) coiling of *Trichoderma harzianum* ATCC 56678 hyphae around *A. brassicicola* mycelium. Bar = 70  $\mu$ m; (b) hyphae of *T. harzianum* 420 growing towards hyphae of *A. raphani*. Bar = 80  $\mu$ m; (c) parallel growth of hyphae of *Fusarium* sp. along *A. brassicicola* hyphae. Bar = 25  $\mu$ m; (d) scanning electron micrographs of *Chaetomium globosum* hyphae coiling around conidia of *A. brassicicola*. Bar = 35  $\mu$ m; (e) scanning electron micrographs of *C. globosum* hyphae coiling around hyphae of *A. brassicicola*. Bar = 7  $\mu$ m; (f) scanning electron micrographs of *C. globosum* hyphae coiling around *A. raphani* conidia. Bar = 25  $\mu$ m; and (g) reaction zones (arrows) of *A. brassicicola* under the stimulus of *C. globosum*. Bar = 25  $\mu$ m (Courtesy: Vannacci and Harman, 1987).





Three to four sprays of dithiocarbamate fungicides, copper oxychloride and Bordeaux mixture have been found effective for the control of alternaria disease of rapeseed-mustard (Kolte, 1985b). The time of first spray application and the interval and number of sprays would depend upon the type of crop. In *B. rapa* and *B. juncea* crops in India, a significant control of the disease is achieved if the first spray is given about 60–75 days after sowing; 2 to 4 more sprays at 10 to 15 days intervals, depending on the maturity of the crop, increases the control (Kaushik *et al.*, 1983, 1984; Kolte, 1985b; Tripathi *et al.*, 1987). The economics, in relation to number of sprays, have been calculated at Hisar, India for the most effective five fungicides in rapeseed-mustard. Four sprays of each of Dithane M-45, Dithane Z-78, Difolatan, Duter and Blitox-50 are required to minimize the disease and increase the yield. However, the maximum net profit is obtained with four sprays of Dithane M-45 starting after 30–45 days of sowing at an interval of 15 days (Tables 17,18) (Kaushik *et al.*, 1983, 1984; Saharan and Chand, 1988; Tripathi *et al.*, 1987).

Integrated control of *Alternaria* and aphid population is obtained with a mixture of three fungicides, Dithane M-45, Difolatan, Blitox, and three insecticides, Rogar, Metasystox and Dimecron (Table 19). The maximum net profit is obtained with sprays of Difolatan + Metasystox followed by Difolatan + Dimecron, Blitox + Metasystox and Dithane M-45 + Metasystox (Saharan, 1992a; Tripathi *et al.*, 1985).

According to Thind and Jhooty (1988) Captafol (0.2%) provides maximum disease control and persists longer on all brassicas. Mancozeb, Zineb and Copper oxychloride at 0.3% exhibits highly differential efficacy against this disease on test brassicas. In mustard, Shivpuri *et al.* (1988) obtained best control of alternaria blight and a significant increase in yield with Rovral followed by Captafol and Dithane M-45 (0.2%). Sinha and Prasad (1989) also found Captafol best, followed by Dithane M-45, for the control of alternaria blight of cauliflower to provide maximum yield. Similarly, Captafol applied at 0.2% (W/V) four times at an interval of 15 days also reduces disease intensity from 69.8% to 15.6% in *B. juncea*, from 79.6% to 20.5% in *B. rapa* var. yellow sarson, and from 74.6% to 19.6% in *B. rapa* var. brown sarson; a significant increase in yield is also obtained (Saharan, 1991). The optimum time for application of fungicides to control alternaria disease of *B. juncea* has been found to be 30–45 days after sowing. Fungicidal sprays applied 60 days after sowing are unable to arrest the disease.

In Bangladesh, among the three fungicides tested, Dithane M-45 proved to be the best for controlling alternaria blight of *B. rapa* var. Sonari Sarisha. Maximum reduction in disease intensity and maximum increase in yield is obtained with 7 sprays starting at 30 days after seeding. Disease control by Trimiltox forte and Cuprovit is also satisfactory (Meah *et al.*, 1988).

In England, iprodione (0.5 Kg a.i./ha) or procymidone (0.75 Kg a.i./ha) has been shown to protect winter oilseed rape and turnip rape against *A. brassicae* for a period of up to 7 weeks following application at 95% petal fall (GS 4.9) (Evans and Gladders, 1981; Evans *et al.*, 1983, 1984, 1988; Gladders and Rhodes, 1985). Cox *et al.* (1981, 1983) in the U.K. and France also reported good control of this disease with a single spray application of iprodione at the early pod stage. Control is obtain-



Table 17. Optimum time for spraying Difolatan (2 g Product/L water) for the control of alternaria leaf blight of raya at different locations in 1979-80 (Saharan, 1984)

| Sprays after sowing (days) | HISAR                 |                 | LUDHIANA              |                 | KANPUR                |                 |
|----------------------------|-----------------------|-----------------|-----------------------|-----------------|-----------------------|-----------------|
|                            | Disease intensity (%) | Yield/ha (Qtls) | Disease intensity (%) | Yield/ha (Qtls) | Disease intensity (%) | Yield/ha (Qtls) |
| 30 - 45                    | 33.7                  | 21.6            | 46.0                  | 19.0            | 20.4                  | 8.4             |
| 60                         | 42.9                  | 19.7            | 46.4                  | 17.8            | 24.2                  | 7.7             |
| 75                         | 50.5                  | 19.5            | 53.4                  | 19.9            | 30.7                  | 7.3             |
| 82                         | 57.8                  | 19.2            | --                    | --              | 36.7                  | 6.6             |
| 90                         | 54.7                  | 19.8            | 62.3                  | 17.8            | 43.3                  | 6.4             |
| 105                        | --                    | --              | 73.8                  | 17.0            | --                    | --              |
| 120                        | --                    | --              | 78.9                  | 14.7            | --                    | --              |
| Control                    | 63.4                  | 18.3            | 84.4                  | 9.1             | 46.9                  | 6.1             |

ed with high volume sprays and with volumes as low as 22 L/ha applied by aircraft. Flowable formulations are superior to wettable powder (Humpherson-Jones *et al.*, 1981). Seed yield increase of up to 20–30% together with low residual levels of inoculum on the seed has been reported following application of iprodione (Ogilvy, 1984). Two sprays of Prochloraz at 500 g a.i./ha, the first at stem extension and second at mid late flowering, have also proved effective in controlling this disease in winter oilseed rape together with a significant increase in yield (Marshall and Harris, 1984). Procymidone, iprodione, and Prochloraz fungicides have also been reported effective against *Alternaria* spp. on winter oilseed rape in Poland (Bonin and Fratzczak, 1987), and chinese cabbage in Denmark (Anonymous, 1988).

In Czechoslovakia, *A. brassicae* and *A. brassicicola* on cauliflower are successfully controlled and yield increased by 2–22 times after spraying with a mixture of Thiram + Benlate alternated with Mancozeb + insecticide (Kudela *et al.*, 1978).

For the control of alternaria blight of radish seed crop, Dithane-M-45 (0.25%) proved most effective, followed by Bordeaux mixture (0.4%) in increasing seed weight, improving seed germination and in reducing seed infection (Hussaini and Singh, 1989). However, Mondal *et al.* (1989) found Rovral (0.2%) best followed by Dithane M-45, Dithane Z-78 and Captan for the control of alternaria blight in radish.

In glasshouse tests in the UK, Channon (1970) found Propineb and Mancozeb (0.2%) sprays consistently effective against *A. brassicicola* on cabbage. Similarly, three sprays of iprodione at 0.5–1 Kg a.i./ha rate, applied at 3 week intervals from the young green pod stage until cutting, controls pod infection of *A. brassicicola*, and increases yields (Humpherson-Jones and Maude, 1982b). In India, Singh *et al.* (1989) found three sprays of Mancozeb, Cuman L and Zineb best for the



Table 18. Efficacy and economics of fungicidal spray on alternaria leaf spot of raya at Hisar in 1977-78 (Saharan, 1984)

| Fungicides   | No. of spray | %<br>Disease intensity | Yield/ha<br>(Qtls) | Net profit/ha<br>(Rupees) |
|--------------|--------------|------------------------|--------------------|---------------------------|
| Dithane M-45 | 1.           | 47                     | 12                 | 53                        |
|              | 2.           | 45                     | 13                 | 72                        |
|              | 3.           | 40                     | 14                 | 372                       |
|              | 4.           | 29                     | 15                 | 803                       |
| Dithane Z-78 | 1.           | 51                     | 13                 | 212                       |
|              | 2.           | 42                     | 13                 | 70                        |
|              | 3.           | 40                     | 14                 | 312                       |
|              | 4.           | 33                     | 15                 | 751                       |
| Difolatan    | 1.           | 42                     | 12                 | 12                        |
|              | 2.           | 37                     | 13                 | 152                       |
|              | 3.           | 32                     | 15                 | 639                       |
|              | 4.           | 22                     | 16                 | 792                       |
| Duter        | 1.           | 50                     | 13                 | 248                       |
|              | 2.           | 38                     | 14                 | 444                       |
|              | 3.           | 33                     | 14                 | 451                       |
|              | 4.           | 28                     | 16                 | 748                       |
| Blitox-50    | 1.           | 53                     | 13                 | 211                       |
|              | 2.           | 47                     | 14                 | 357                       |
|              | 3.           | 43                     | 13                 | 187                       |
|              | 4.           | 32                     | 15                 | 748                       |
| Control      |              | 62                     | 12                 | ---                       |

control of *A. brassicicola* leaf spot of cabbage.

Storage rot of white cabbage due to *A. brassicicola* can be effectively controlled with 5% iprodione mixed in talc powder (Kear *et al.*, 1977). Similarly, a post-harvest iprodione drench on stored winter cabbage controls *Alternaria* spp. (Geeson and Browne, 1979). On the other hand Tasca and Trandaf (1984) reported preharvest treatment of cabbage with Mancozeb (0.2 %) or Folpet (0.2%) for protection from *A. brassicae* storage rots. Ronilan-smoke, a vinclozolin formulation used as smoke pellets, in *Brassica* storage rooms also controls *A. brassicae* (Jennrich, 1985).

Table 19. Effect of fungicide and insecticide mixture on alternaria leaf spot and aphid population on brown sarson (BSH-1) at Hisar in 1977-78 (Saharan, 1984)

| Mixture of fungicide and insecticide | Aphid Population/<br>10 twigs | %<br>Disease intensity | Yield/ha<br>(Qtls.) | Net profit/ha<br>(Rupees) |
|--------------------------------------|-------------------------------|------------------------|---------------------|---------------------------|
| Dithane M-45 + Rogor                 | 72                            | 36                     | 9.0                 | 875                       |
| Dithane M-45 + Metasystox            | 57                            | 25                     | 9.7                 | 1088                      |
| Dithane M-45 + Dimecron              | 75                            | 42                     | 8.8                 | 766                       |
| Difolatan + Rogor                    | 75                            | 31                     | 8.4                 | 459                       |
| Difolatan + Metasystox               | 62                            | 17                     | 10.7                | 1285                      |
| Difolatan + Dimecron                 | 72                            | 33                     | 10.4                | 1279                      |
| Blitox + Metasystox                  | 70                            | 48                     | 9.9                 | 1151                      |
| Blitox + Rogor                       | 107                           | 49                     | 8.8                 | 748                       |
| Blitox + Dimecron                    | 67                            | 56                     | 8.9                 | 836                       |
| Control                              | 820                           | 72                     | 5.6                 | --                        |
| C.D. at 5 %                          | 6.8                           | 6.6                    |                     |                           |

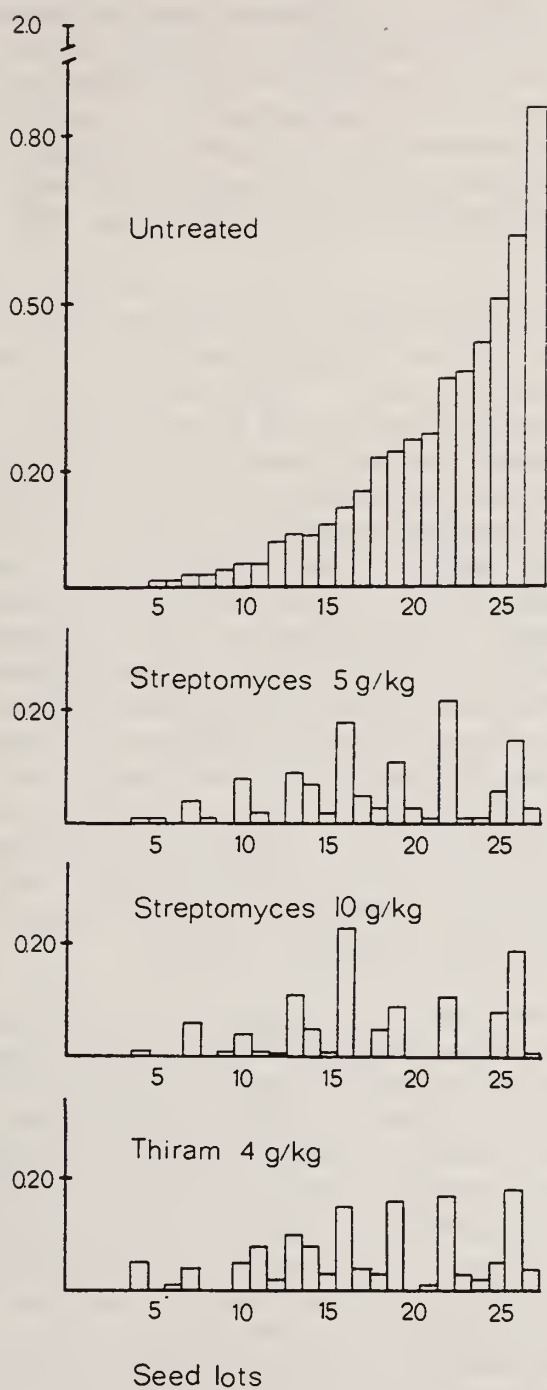
Foliar diseases of brussels sprouts in the UK are effectively controlled with mixtures of iprodione, triademefon/carbendazim and metalaxyl/Mancozeb. The timing of the sprays is more important than the number of applications. Two sprays, one in August and one in September, are enough to reduce fungicide input (Brokenshire, 1987; Davies *et al.*, 1986).

#### d. Biological Control

i) **Plant extracts as fungitoxicants:** Essential oil from the roots of radish inhibits (1:2500) *A. brassicae* (Nehrash, 1961). The deproteinized leaf extracts of *Acacia nilotica*, *Enicostema hyssopifolium*, *Mimosa hamata*, and *Vitis vinifera* have shown fungistatic activity against *A. brassicicola* (Umalkar *et al.*, 1977). The extracts prepared from the leaves of *Lawsonia alba*, roots of *Datura stramonium* and inflorescence of *Mentha piperita* have fungitoxic activity against *A. brassicae* isolated from cauliflower leaves. Extracts of the ferns *Adiantum caudatum*, *Diplazium esculentum* and *Pteris vittata* reduces growth and germination of *A. brassicicola* (Yasmeen and Saxena, 1990; Ahmed and Agnihotri, 1977).

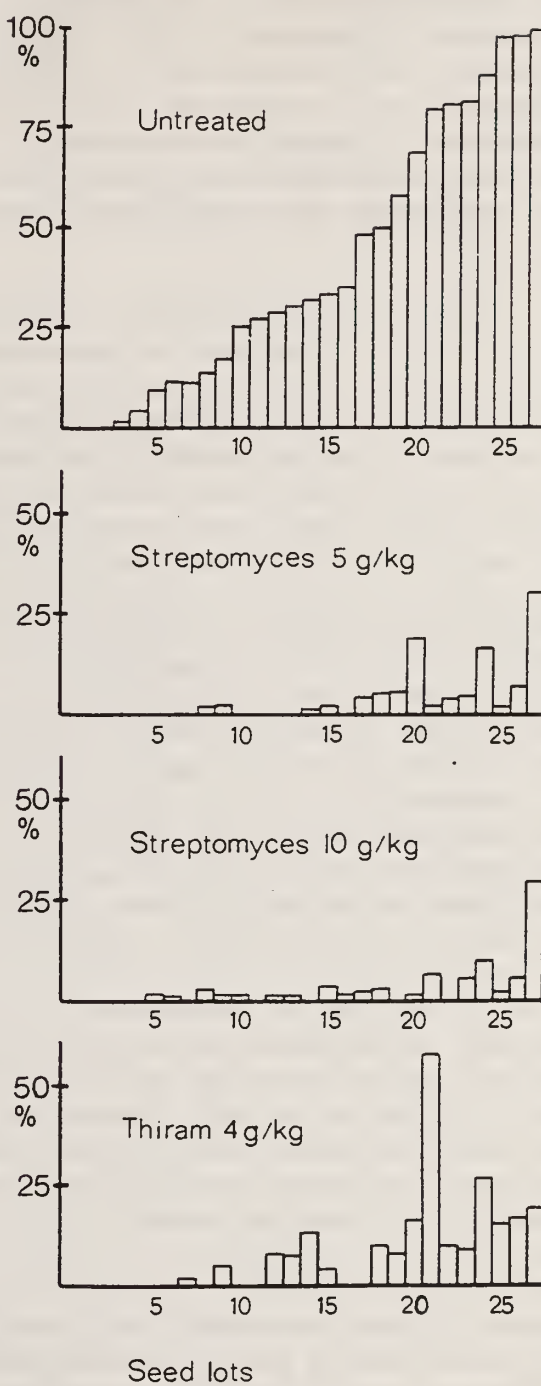
ii) **Antagonists for biocontrol:** Saprophytic phylloplane fungi such as *Aureobasidium pullulans* and *Epicoecum nigrum* are pathogenic to *A. brassicicola* (Pace and

Disease index



a

Alternaria content of seeds



b

Fig. 27. The biological control of (a) seed-borne *Alternaria brassicicola*; and (b) *A. brassicicola* content of seedlings grown from naturally-infected *Brassica* seed with a powdery preparation of *Streptomyces* sp. (Courtesy: Tahvonnen and Avikainen, 1987).



Campbell, 1974). The *Verticillium*-state of *Nectria inventa* Pethybridge, a destructive mycoparasite, is one of the dominant phylloplane fungi of rapeseed (Tsuneda and Skoropad, 1978a). Amongst the leaf surface mycoflora, the most antagonistic fungi are *E. purpurascens*, *A. pullulans* and *Cladosporium cladosporioides* in case of *A. brassicae*. The metabolites of *Acremonium roseogriseum*, *Aspergillus terreus* and *C. cladosporioides* inhibits *A. brassicae*. The most significant effects are observed when the spores of the leaf surface fungi and their metabolites are sprayed prior to inoculation of pathogen on leaves (Rai and Singh, 1980).

Pre-treatment application with the spore suspension of *Streptomyces rochei* or its diffusate results in a marked reduction of leaf blight intensity caused by *A. brassicae* and *A. brassicicola* on brown sarson (*B. rapa* var. *dichotoma*) (Sharma and Gupta, 1978a,b, 1979). With the decline in the population of *S. rochei* there is a rise in the population of *Alternaria* from December onwards indicating the possibility of antagonism. Several factors, such as climatic conditions, age of the host plant, maturity of the leaves and variety of the host have profound influence on the deposition of conidia of both the pathogens and *S. rochei* (Sharma and Gupta, 1980). Jayant and Sinha (1981) reported that *S. hygrosopicus* is strongly antagonistic to *A. brassicae* and *A. brassicicola*. When the culture filtrate is sprayed over the sarson plants, a week before or a week after spraying the spore suspension of *A. brassicae* and *A. brassicicola*, germination and disease development is reduced. Whenever the spore population of *Streptomyces arabicus* is higher on leaves of yellow sarson and taramira the population of conidia of *A. brassicae* and *A. brassicicola* declines. The population of antagonistic *S. arabicus* is higher in the young leaves as compared to old ones (Sharma *et al.*, 1984). The antifungal substance in the diffusate of the antagonist is thermolabile (Sharma *et al.*, 1985a). A pigmented and xylose-utilizing strain of *S. bobili* is found to be active against *A. brassicae*, *A. brassicicola* and *A. raphani* (Sharma and Sinha, 1989).

An isolate of *Streptomyces* spp. obtained from light-coloured, Finnish Sphagnum horticultural peat has proved effective biological control agent against plant pathogens (Tahvonen, 1982a,b). Treating cauliflower seeds with *Trichoderma viride* and *Streptomyces* spp. isolates inhibits or reduces damping-off caused by *A. brassicicola* (Tahvonen, 1982b, 1988). Seed dressing with Mycostop, a powdery formulation prepared from spores and mycelium of *Streptomyces*, is 80–90% successful in controlling damping-off from seeds artificially infected with *A. brassicicola*. The dressing remains effective on seeds stored under dry conditions for 5-6 weeks, but subsequently decreases slowly. *Streptomyces* dressing controls in a manner comparable to chemical dressing with Thiram, preventing damping-off caused by *Alternaria* fungi in seedlings which are grown from commercial seed lots of different origin (Fig. 27; Tahvonen, 1985; Tahvonen and Avikainen, 1987). White *et al.* (1990) reported that Mycostop, a biofungicide based on a selected *S. griseoviridis* isolate from Finnish Sphagnum peat, introduced either by seed treatment or soil treatment, controls *A. brassicicola* of cauliflower and cabbage (Fig. 25).

Brassicaceous seeds treated with either *Gliocladium virens*-19, *Trichoderma harzianum*-22, *T. harzianum*-50, *Pencillium corylophilum*-36 and *P.*

*oxalicum*-76 have a significantly higher emergence of healthy seedlings. The hyphae of antagonistic fungi are able to adhere to conidia or coil around or penetrate germ tubes or hyphae of *A. brassicicola*. Conidia of *A. brassicicola* shrivel and plasmolyze in the presence of antagonistic fungi (Fig. 28; Wu and Lu, 1984b). For biocontrol of seed-borne *A. raphani* and *A. brassicicola* of radish antagonists like *Chaetomium globosum*, *T. harzianum*, *T. koningii* and *Fusarium* spp.-treated plants show consistently increased numbers of healthy seedlings (Vannacci and Harman, 1987). Wu and Lu (1984b) found *Trichoderma*, *Gliocladium* and *Penicillium* spp. parasitizing *A. brassicicola*. The application of *A. alternata*, prior to inoculation with *A. brassicae*, reduces the level of *A. brassicae* on oilseed rape by about 60% and subsequent to inoculation by about 26% (Mee, 1991). In Poland, Madej (1986) reported *Gonatobotrys simplex* as a hyperparasite of *A. brassicae*.

**iii) Mechanism of biocontrol:** Antagonistic mechanisms of bio-control has been studied in a few selected host-parasite systems. The parasitism of *A. brassicae* by the *Verticillium*-state of *Nectria inventa* occurs either by penetration or contact without penetration. Parasitic hyphae induce abnormal responses in host cells upon contact. A reaction consisting largely of an electron- dense transparent matrix and dispersed tubule-like electron-dense material develops between the cell wall and the invaginated plasma membrane. The tubule-like elements subsequently aggregate to form electron-dense deposits below the cell wall. The affected cell forms a septal plug, accumulates membranes and finally degenerates. Hyphae of *N. inventa* penetrate the conidial cells of *A. brassicae* mainly by a process which appears primarily enzymic in nature. The cytoplasm of the penetrated cell becomes progressively less dense and the cell eventually appears empty (Tsuneda, 1977; Tsuneda and Skoropad, 1980; Tsuneda *et al.*, 1976). Penetration of *A. brassicae* hyphae causes separation of the cells, penetration of conidia occurs most frequently at the septum or at the basal pore in juvenile conidia (Tsuneda and Skoropad, 1977a,b). Conidia of *N. inventa* requires at least 24 h to initiate germination and 4 days to parasitize *A. brassicae* on intact leaves (Figs. 29–33; Tsuneda & Skoropad, 1978b). Conidia of *A. brassicae* leak various amino acids and sugars when they are exposed to an alternate dry wet condition, the longer the drying period, the larger the amount of leakage. Among the amino acids glutamine is exuded in the largest amount followed by aspartic acid and glutamic acid. The sugar fraction consists mainly of glucose and fructose. These leaked nutrients stimulate germination and growth of *N. inventa*. As a result the germination of *Alternaria* conidia is drastically reduced to less than 5% and they are eventually destroyed by *N. inventa* (Tsuneda and Skoropad, 1978c).

Many antagonistic species produce antibiotic substances which have the ability to produce enzymes causing the lysis of cell wall components of the pathogenic fungus. This helps the antagonist to penetrate the host hypha and grow on it as a hyperparasite (Tapio and Pohto-Lahdenpera, 1989).

#### **e. Host Resistance**

Sources of resistance in different oil yielding and vegetable brassicaceous hosts have been identified, which can be incorporated through conventional and biotechnological techniques under suitable agronomically sound yield and quality



bases to be effective against *Alternaria* spp. However, exploitation of this information to manage *Alternaria* disease under field conditions needs much more emphasis.



Fig. 28. The effectiveness of antagonistic fungi against *Alternaria brassicicola*: (a) *Penicillium corylophilum*-36, and (b) *Trichoderma harzianum*-22 coiled around the conidia on agar medium; (c) *T. harzianum*-22 coiled around a conidium on a cabbage seed; and (d) *P. citrinum* coiled around the conidial germ tube on a cabbage root (Courtesy: Wu and Lu, 1984).



#### 14. FUTURE STRATEGIES AND PRIORITIES IN THE MANAGEMENT OF ALTERNARIA DISEASES OF CRUCIFERS

With the increasing demand of a growing global population, there has been a significant change in cropping patterns of edible and industrial oil-yielding crucifers as well as vegetables. In the absence of host resistance, intensive cultivation has resulted in perpetuation, build-up, and dissemination of *Alternaria* species on crucifers in areas where these crops are grown. The main factors responsible for the increase of inoculum of pathogenic *Alternaria* to the present level in-

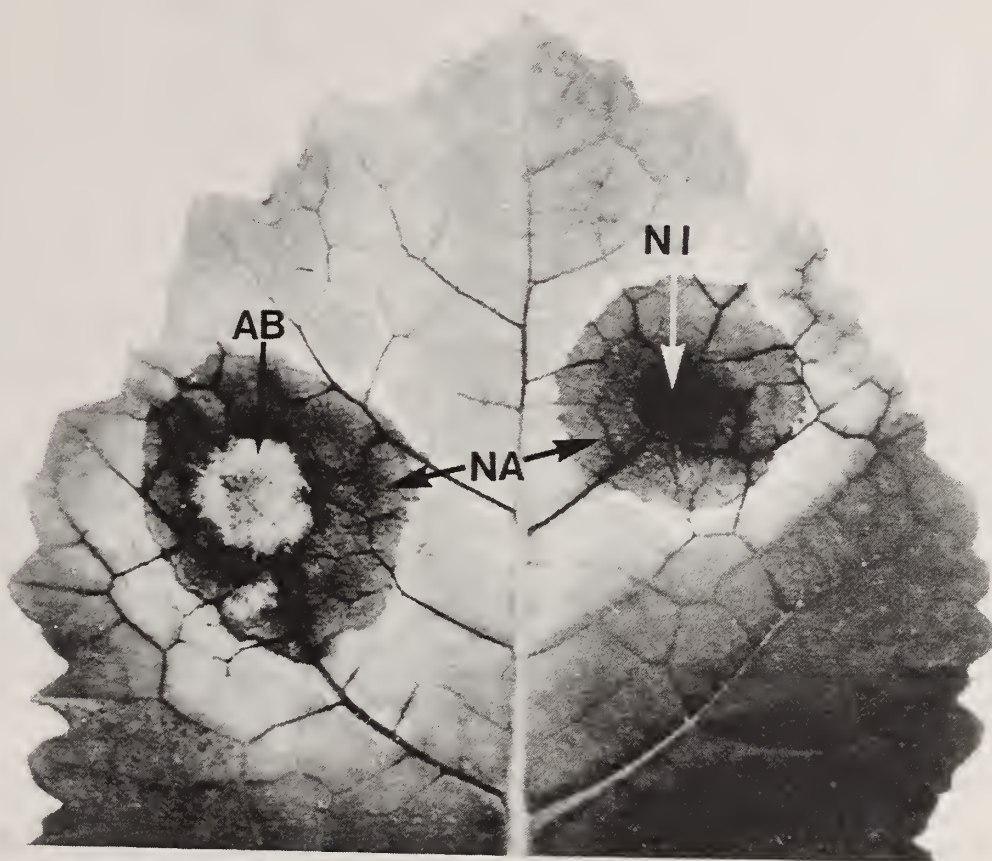


Fig. 29. Excised non-dried rapeseed leaf inoculated with *Alternaria brassicae* alone (left half of leaf) and with the mixture of *A. brassicae* and *Nectria inventa* (right half of leaf) at 1 week after inoculation. In the necrotic areas (NA) caused by *A. brassicae*, note the presence of a white mycelial mass of *A. brassicae* with conidia (AB) on the left half and its absence on the right half of the leaf with abundant conidia of *N. inventa* (NI). x 1.5 (Courtesy: Tsuneda and Skoropad, 1978b).

clude the lack of genetic sources of resistance, cultivation of susceptible crops with high plant density, use of irrigation with high rates of nitrogen fertilizers, contiguous areas under monoculture, staggered sowing dates, poor weed management, and poor plant protection strategies. The information gathered in this

monograph on *Alternaria* diseases of crucifers indicates that some gaps still exist in our comprehensive understanding of various dimensions of *Alternaria*-host pathosystem; some of these are highlighted below:

**a. Disease Epidemiology**

Factors governing disease development and consequent progression are not completely understood. Deeper insight with regard to various epidemiological aspects will help to develop strategies to curb the progression of these potential diseases. There is an urgent need to carry out intensive investigation with regard to the environmental parameters responsible for causation and spread of diseases under field conditions. Multilocal trials with staggering dates of planting could be helpful in analyzing disease development in relation to environment by computing disease progression at regular intervals. These efforts could be undertaken to study various "Disease Prediction Models" (geophytopathological, bioclimatic, simulation system analysis, etc.) which could play a meaningful role in developing an effective disease control strategy.

**b. Physiological Specialization**

Identification and standardization of host differentials is a fundamental prerequisite to the gathering of meaningful information on races/pathotypes. This is a high priority area of investigation.

**c. Genetics of Resistance**

Although we have some information on genetic sources of resistance, the information on the nature and inheritance of resistance are still lacking. The results of this area of investigation are an important prerequisite to incorporating stable and effective sources of resistance in breeding programs. In diseases caused by *Alternaria* species where major gene resistance is not known, the potential of minor genes/horizontal resistance should be exploited.

**d. Disease Control Strategy**

Some useful information is available on chemical control of various *Alternaria* diseases, however, much more is needed in order to develop a viable disease control strategy under field conditions. Efforts should also continue to search for low cost effective chemicals which can provide economically significant disease control.

Control of *Alternaria* diseases in rapeseed-mustard by microbial antagonists should also be explored. Seed treatment with *Trichoderma* spp., *Gliocladium* spp., *Penicillium* spp., and Mycostop-like preparations of *Streptomyces* spp. should be further investigated to determine their level of control and economic feasibility under field conditions. Similarly, control by foliar applications of *Streptomyces rochei*, *S. hirsutus*, *S. arabicus* and *Nectria inventa* should also be evaluated under field conditions.



### e. Integrated Management

No single method or approach can be effective and economical in dealing with all biological systems. Therefore, all means of plant disease control including chemical, biological, cultural and host resistance should be integrated to manage *Alternaria* diseases of crucifers.

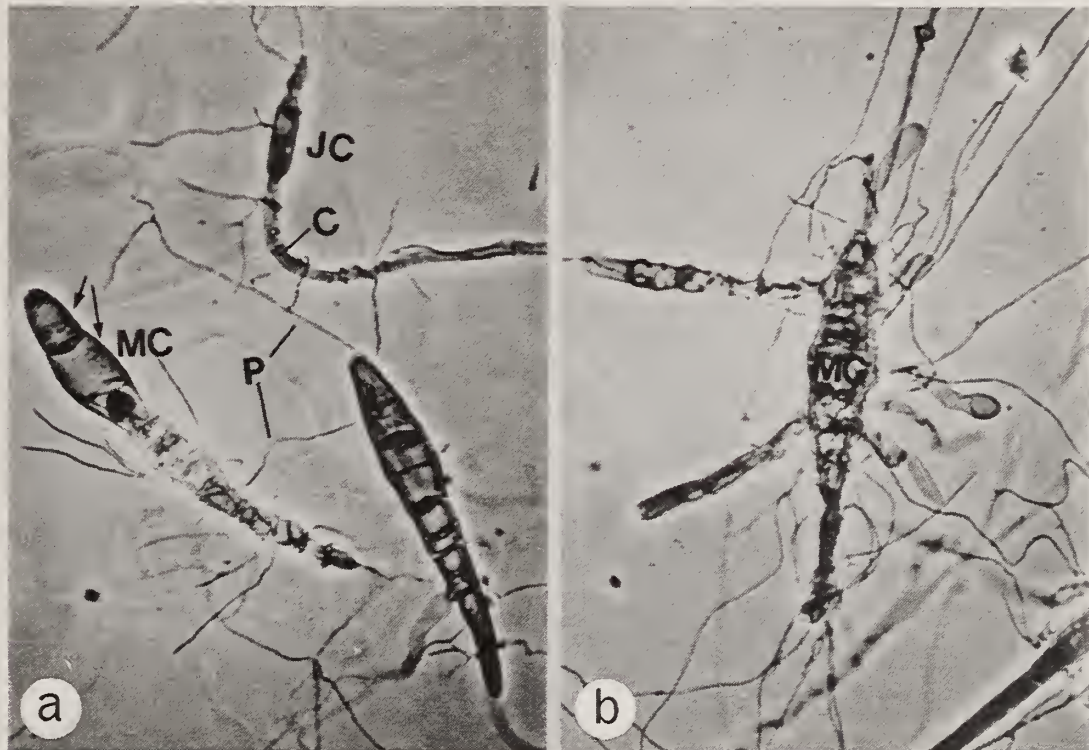


Fig. 30. Phase contrast light micrographs of the conidia of *Alternaria brassicae* parasitized by *Nectria inventa*: (a) healthy-appearing conidium and infected mature and juvenile conidia. Note noninfected cells (arrows) in the heavily infected conidium. x 1,200; and (b) profuse growth of parasitic hyphae around a host conidium. x 1,200. Legend: P=parasite; C=host conidiophore; MC=mature host conidium; and JC=juvenile host conidium (Courtesy: Tsuneda, Skoropad and Tewari, 1976).



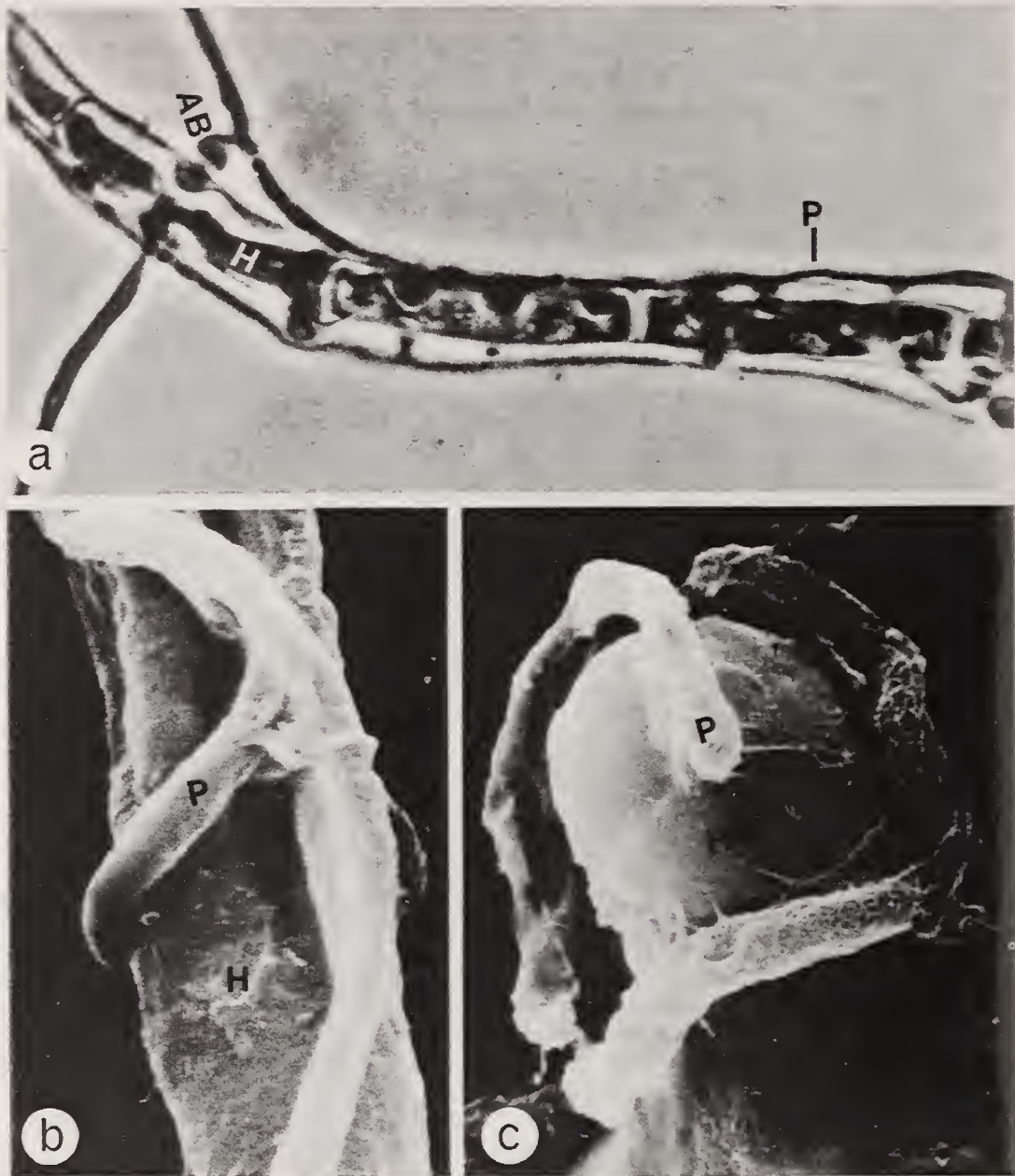


Fig. 31. Hyphae of *Alternaria brassicae* parasitized by *Nectria inventa*: (a) light micrograph of parasite hyphae parallel to a host hypha. Note the swollen appressorium-like body of the parasite. x 4,300; and (b,c) scanning electron micrographs of the parasite coiling around host hyphae. x 15,000. Legend: P=parasite; H=host; AB=appressorium-like body of parasite; and HB=hyphal branch of the host (Courtesy: Tsuneda, Skoropad and Tewari, 1976).



Fig. 32. Scanning electron micrographs of mycoparasite *Nectria inventa* hyphae growing on *Alternaria brassicae*: (a) parasite hyphae occurring predominantly in the septal area (arrows) and the basal portions of the germ tubes (G) of a host conidium. x 1,800; (b) appressorium-like bodies (A) formed on the host conidium. Note presence of adhesive material under these bodies. x 11,000; and (c) appressorium-like body (A) with fibrous adhesive material (arrow) formed on host hypha (H). x 25,000 (Courtesy: Tsuneda and Skoropad, 1977a).



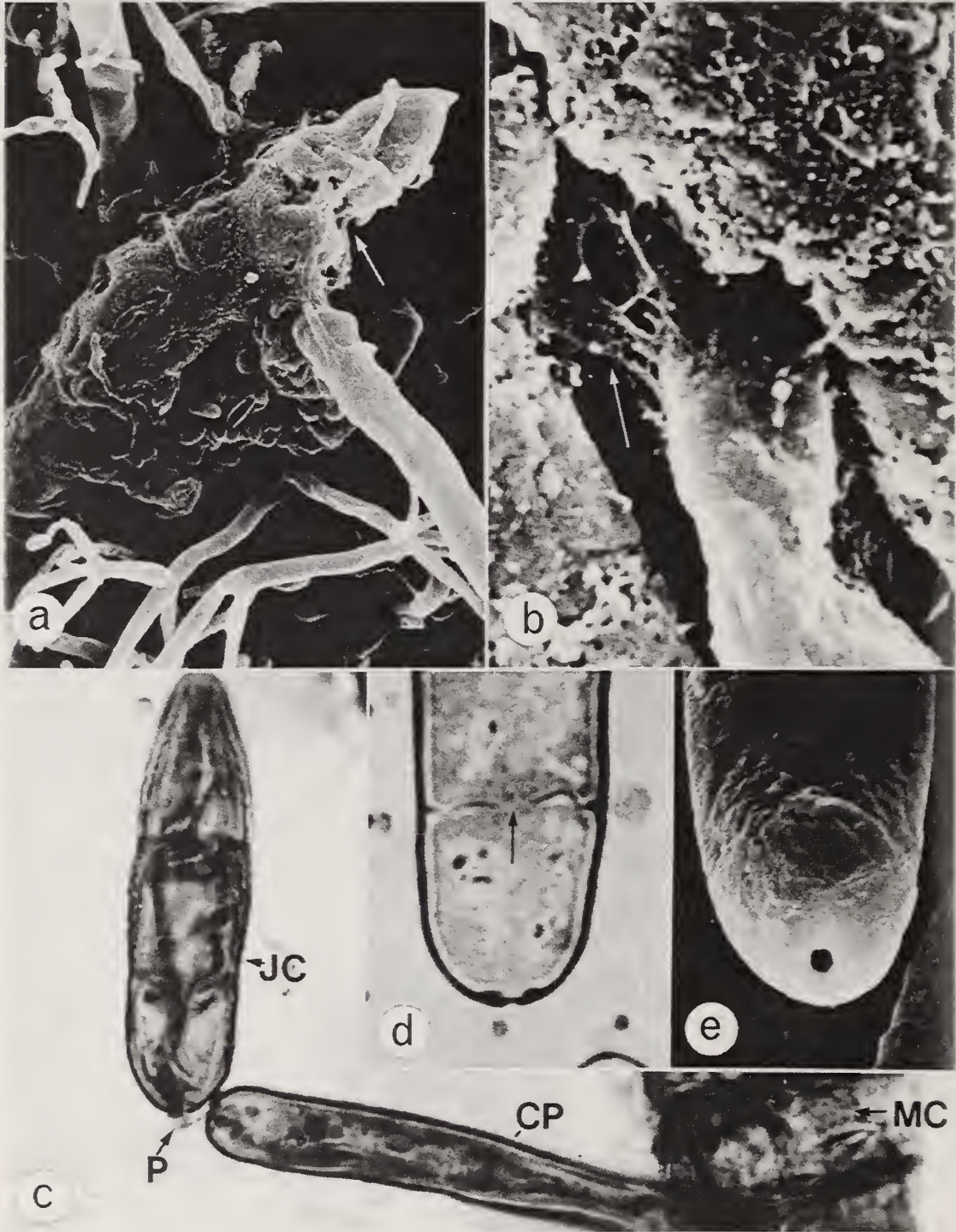




Fig. 33. The *Alternaria brassicae*-*Nectria inventa* host parasite interface: (a) mature conidium of *A. brassicae* penetrated by hyphae of *N. inventa*. Note collapsed cell wall of the conidium (arrow). x 1,800; (b) enlarged view of penetration site. A large hole develops in the wall of the host cell, and a meshwork of material appears at the penetration site (arrow). x 27,000; (c) penetration of a juvenile conidium (JC) of *A. brassicae* by *N. inventa* (P). The host conidium is penetrated through the basal pore. MC, infected cell of a mature conidium. CP, conidiophore produced by the mature conidium. x 2,500; (d) light micrograph of a thin section of a normal juvenile conidium of *A. brassicae* showing the basal pore and septal pore (arrow). x 2,800; and (e) scanning electron micrograph of a basal pore in a juvenile conidium of *A. brassicae*. x 3,500 (Courtesy: Tsuneda and Skoropad, 1977a).

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**Incubation Period:** 57, 59.

**India:** 8, 10-16, 40, 50, 53, 85, 87, 92, 93.

**Infection Grades:** 23.

**Infection of Host:** 50, 51, 57, 63.

**Infection Rate:** 57, 68.

**Inheritance of Resistance:** 68.

**Inhibition of Spore Germination:** 44, 70, 71, 87.

**Incubation Method:** 35.

**Inoculation Technique:** 79-84.

**Inoculum:** 22, 41, 61, 80.

**Insecticides:** 90, 92, 95.

**Integrated Control:** 85, 92, 102.

**In-vitro Study:** 65, 82-84, 87.

**Ireland:** 10, 11, 14-16.

**Irrigation:** 57.

**Isolation:** 35.

**Isolates:** 35.

**Italy:** 11, 12, 15.

**Jamaica:** 11, 15.

**Japan:** 11, 12.

**Kale:** 5, 15, 46, 48.

**Kenya:** 11, 13.

**Kohlrabi:** 13, 15, 46, 48.

**Latent Period:** 41, 57, 59, 68.

**Latvian SSR:** 14.

**Leaf Age:** 44, 68.

**Leaf Extracts:** 44, 95.

**Leaf Exudates:** 44, 84.

**Leaf Surface Mycoflora:** 97.

**Leaf Wetness:** 44, 53, 55.

**Libya:** 11.

***Lepidium* Species:** 14.

**Light and Darkness:** 39.

**Light Intensity:** 44, 55.

**Losses in Oil Content:** 16-20.

***Lunaria annua*:** 13, 14.

***L. rediviva*:** 14.

***Macrosporium* Species:** 12, 25, 26, 30.

**Malawi:** 11.

**Malaya:** 11, 15.

**Malaysia:** 11.

***Mauritious*:** 10, 15.

***Matthiola incana*:** 8, 12, 13.

**Metabolites:** 41, 52, 68, 84, 97.

**Mexico:** 14.

**Microsclerotia:** 41-43, 46, 51.

**Mode of Infection:** 51.

**Morocco:** 11, 12.

**Mozambique:** 11.

**Mutation:** 74.

**Mycelium:** 26-32, 39, 40, 44, 46, 51, 63.

**Mycoparasitism:** 97.



**Mycostop:** 87, 97, 101.

**Necrosis:** 52, 68, 76.

***Nectria inventa*:** 97, 98, 100, 102-106.

**Nepal:** 10.

**Netherlands:** 11.

**New Caledonia:** 13.

**New Guinea:** 11.

**New South Wales:** 45, 86.

**New Zealand:** 11, 13, 15.

**Nicaragua:** 11, 14.

**Nigeria:** 11.

**Nitrogen Source in the Medium:** 36.

**North Caucasus:** 12.

**Norway:** 11, 13.

**Nuclei:** 61, 62, 66, 67.

**Nucleic Acid:** 62.

**Nutrition:** 55, 57.

**Nyasaland:** 13, 15.

**Oil Content:** 17-19.

**Oilseed Rape:** 15, 40, 55, 64, 65, 73, 74, 92, 93, 98.

**Ovule Culture:** 84.

**Oxalic Acid:** 71.

**Pakistan:** 11.

**Pathogen:** 25.

**Pathogen Morphology:** 26.

**Pathogen Perpetuation:** 39-41, 46.

**Pathogen Taxonomy:** 26.

**Pathogenesis:** 34, 35, 38, 51, 52, 63.

**Pathogenic Isolate:** 35.

**Pathogenic Variation:** 34, 35.

**Pathotype:** 36.

**Pectinases:** 63.

**Penetration:** 98.

**Phenolic Compounds:** 69.

**Philippines:** 11, 12.

**Phospholipids:** 62, 63.

**Phylloplane Microflora:** 95, 97.

**Physiological Specialization:** 34, 36, 101.

**Phytoalexins:** 64, 70, 71.

**Phytotoxin:** 68, 76.

**Plant Age:** 68, 97.

**Plant Growth Stage:** 69, 79-81.

**Poland:** 5, 11, 14, 15, 98.

**Pollen Selection:** 81.

**Protein Content:** 16-20.

***Pseudostemphylium*:** 26.

**Radish:** 12-16, 35, 40, 41, 45, 46, 49, 50, 55, 57, 64, 87, 93, 95, 98.

**Radish Root Extract:** 64, 82, 95.

**Rainfall:** 53.

**Rape:** 14-16, 20, 45, 46, 48, 49, 52, 53, 57, 71, 92, 97.

**Rapeseed-Mustard:** 6, 10, 16, 35, 40, 46, 48, 49, 52, 53, 59, 92, 101.

***Raphanus sativus*:** 5, 36.

**References:** 106-145.

**Regression Analysis:** 22.

**Relative Humidity Effect:** 37, 39, 44, 52, 53, 55, 79.

**Remote Sensing:** 24.

**Resistance:** 22, 36, 57, 59, 65, 68-72, 74, 78-80, 98.

**Resistant Variety:** 59, 74.

**Rhodesia:** 11.

***Rhopalidium*:** 26.

**Root and Foot Rot:** 8.

**Romania:** 11, 14.

**Rutabaga:** 9, 20, 46, 48.

**Russia:** 11, 12.

**Sabah:** 11.

**Sanitation:** 85.

**Sarawak:** 15.

**Saudi Arabia:** 11, 15, 16.

**Scotland:** 11, 45.

**Sea Kale:** 5, 46.

**Seed-borne Infection:** 17, 18, 39, 40, 44-46, 50, 55, 82, 83, 86, 90.

**Seed Disorder:** 46.

**Seed Mycoflora:** 46.

**Seed Quality:** 17.

**Seed Transmission:** 40.



**Seed Treatment:** 85, 86, 87, 88, 97.

**Senescence:** 63.

**Sierra Leone:** 11.

***Sinapis* Species:** 14, 68, 74.

**Singapore:** 11, 14.

**Sinigrin:** 37.

***Sisymbrium* Species:** 14.

**Slugs Association:** 53.

**Soil Culture:** 41.

**Somaclonal Variation:** 74, 78.

**Sources of Resistance:** 72-74, 98.

**South Africa:** 11, 45.

**Spain:** 11, 14, 16.

**Spore Dispersal:** 46, 51, 58, 64.

**Spore Germination:** 41, 44, 50, 51, 87.

**Spore Structure and Development:** 39, 61, 62.

**Sporulation:** 35-39, 44, 55, 58, 59, 68, 82, 83.

**Sowing Time Effect:** 57.

**Spraying of Chemicals:** 92-95.

**Sri Lanka:** 11.

**Starch:** 63.

***Stemphylium* spp.:** 25, 26.

**Storage Fungi:** 94.

**Strain:** 34, 35.

***Streptomyces* spp.:** 86-88, 97, 101.

**Structural Formula:** 78.

**Stump Rot:** 9.

**Subcuticular Growth:** 52, 54.

**Sudan:** 11, 13, 15.

**Sugar Beet (*Beta vulgaris*):** 13.

**Sugar Analysis:** 69.

**Susceptibility of the Host:** 36, 57, 59, 69, 79.

**Symptoms:** 6, 77.

**Synonymous:** 5, 30, 32, 33.

**Sweden:** 11, 13, 14, 48, 85.

**Swedes:** 15, 46, 50.

**Switzerland:** 11.

**Taiwan:** 11.

**Tanganyika:** 11, 13.

**Tanzania:** 11.

**Taxonomy:** 26.

**Techniques:** 79-84.

**Temperature Effect:** 37-40, 44, 45, 50-53, 55, 79, 80, 85, 88.

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**Toria:** 6, 46.

**Toxins:** 52, 76-79, 81.

**Toxin Bioassay:** 76, 77.

**Transfer of Resistance:** 74, 76.

***Trichoderma* spp.:** 90, 97-99, 101.

**Trinidad:** 11, 12.

**Turkey:** 11, 15.

**Turnip:** 5, 9, 12-16, 20, 45, 46, 48, 49, 70, 71, 78, 92.

**Uganda:** 11.

**Ultrastructures:** 61.

**United Kingdom:** 12-16, 40, 41, 45, 50, 92, 93, 95.

**U.S.A.:** 5, 11-14, 16.

**U.S.S.R.:** 11, 13-16, 85.

**Variants:** 35, 36.

**Variability in Pathogen:** 34-36.

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**Virulence:** 35, 36, 41.

**Vitamins:** 37.

**Wallflowers:** 11, 41, 49, 50, 84.

**Wax Crystals:** 69-71, 75.

**Weeds as Host:** 39, 41, 51.

**White Cabbage:** 11, 20, 45, 85, 94.

**Wire Stem:** 55.

**Yellow Sarson:** 6, 17, 19, 46, 53, 78, 79, 81, 92, 97.

**Yield Increase:** 87, 92-94.

**Yield Losses:** 16-20.

**Zambia:** 11.







